VOLUME 101 NUMBER 4

THE
BOTANICAL GAZETTE

*June 1940*

# PERMANENT CHANGES OF CHLOROPLASTS INDUCED BY X RAYS IN THE GAMETOPHYTE OF POLYPODIUM AUREUM

LEWIS KNUDSON

(WITH FORTY-SIX FIGURES)

## Introduction

In an investigation on the effects of X rays on growth response and physiological processes in the gametophytic stage of *Polypodium aureum*, certain extraordinary changes were infrequently noted in the chloroplasts of protonema and prothallia derived from spores exposed to relatively high intensities of irradiation. So remarkable were these modifications, and so significant relative to important problems in genetics and physiology, that it appeared desirable to give especial attention to this phase of the problem.

While data on germination and growth were obtained and various extraordinary modifications of protonema and prothallia obtained which will be reported subsequently, the primary problem became a study of these plastids. It was believed that such study could contribute not only to a fuller knowledge of the structure of the plastid, but—if a sufficient number of plants could be obtained—a real contribution could be made to the question of the individuality of the plastid, its continuity from generation to generation, and plastid inheritance. Is the change in the plastid due to genic factors or is it due to cytoplasmic changes and independent of chromosomal

alterations and aberrations? It is recognized, of course, that modification of the plastid may be determined by genic factors, and it has been demonstrated for both animals and plants that the frequency of heritable alterations is markedly increased by X rays and that often associated with these are chromosomal alterations and aberrations (**28, 3, 4**).

Botanists in general are inclined to the view that plastids arise only from pre-existing plastids, so that there is a continuity from one generation to another. WEIER (**30**) has recently summarized the literature on this problem, and concludes that the "most careful work indicates a continuity of the chloroplast." SHARP (**25**), recognizing definite continuity of plastids in certain algae, maintains that the genetic continuity of plastids in general still remains to be proved. WEIER emphasizes the contributions of DE VRIES, RENNER, and others on that type of plastid inheritance which indicates genetic continuity. From the viewpoint of genetic continuity, the work of LANDER (**16**) on the chloroplasts in *Anthoceros* is significantly favorable. STONE (**29**) among recent investigators concludes that plastids arise *de novo*.

It is generally recognized that in meristematic cells the plastids exist as minute bodies, to which a variety of names have been given: mitochondria, chondriosomes, plastid primordia, archiplasts, and proplastids. The last term, suggested by RANDOLPH (**23**), is preferred by BOWEN (**2**), who is able to differentiate these proplastids from similar appearing bodies. In meristematic cells it is not uncommon to find these proplastids grouped about the nucleus, as was observed by RANDOLPH (**23**) in corn. The plastids are in the cytoplasm of course. They grow and divide, and their shape and size may, within limits, vary with nutrition of the cell.

That properties of the cytoplasm are modified by X ray treatment has been demonstrated. It is also probable that such modifications may be associated with the increased ionization and molecular excitation that results from X rays (**4, 5, 6**). With chemical or physical-chemical changes in the cytoplasm itself there might be induced changes in the plastids, or changes in the plastids could occur because of direct action of the X rays. The point to be emphasized is that these changes could occur without involving chromo-

somal behavior. If such changes should persist and if there is continuity of plastids from generation to generation, then mutations in plants could occur without chromosomal changes, especially mutations involving growth and yield.

Entirely aside from the problem of inheritance and plastid individuality, the extraordinary modifications noted in the course of this work seem to offer material of especial value for studies in plastid structure and behavior, and for studies on the relation of size, form, and structure to photosynthetic efficiency. Here in a single species occur distinct types of chloroplasts. The questions arose, will these modifications persist, and can these prothallia be vegetatively increased so that an adequate stock of material will be available for study?

Altogether fifty-one prothallia with marked plastid changes were isolated. Of these only twenty-seven survived, but fortunately these included all the plastid types that had been observed. Fortunately also it proved a relatively simple problem to multiply the prothallia by vegetative reproduction, and at present an abundant stock of material is available. It is the purpose of this first paper to describe the methods employed and to describe the plastid types that have been produced. Subsequent papers will deal with the transmission of these plastids to sporophyte and new gametophyte generations.

## Material and methods

The fern was selected as the plant material because it produces minute spores which are simple in structure. Any effect induced by X rays would be direct and not secondary, as is possible when seeds are used. Furthermore, the spores could be germinated under pure culture conditions and a large number of plants could be grown in a single tube in the entire absence of insects and microorganisms. Any number of plants could be removed from the tube at will for microscopic examination without damaging the remaining plants of the culture. *Polypodium aureum* (*P. glaucum*) (*Phlebodium aureum*), commonly known as the hare's foot fern, was selected because it produces spores at all seasons of the year and in large quantity. This plant is native to southern Florida and tropical America. While the plant is epiphytic, it grows readily in soil. Both sex organs are

borne on the same prothallium. As is well known, the spore is of the gametophytic generation and has therefore the haploid complement of chromosomes.

IRRADIATION.—For irradiation the spores were placed in a small glass container made by cementing a cover glass to a glass cylinder (diameter 1.8 cm.) such as is used in the preparation of a Van Tieghem cell. The bottom of this cell was covered to a depth of about 1 mm. with spores. About ten containers were placed under the X ray tube at a distance of 12.5 cm. from the target. The tube was a Coolidge type 5-30, operated at 78–80 k.v. with a current of 4 milliamperes. Radiation was not filtered. The irradiation was intermittent; that is, the material was irradiated for one minute, with a pause of one minute. Under the conditions described the intensity of the radiation was 1000 r units per minute.

The intensity was measured by means of a Fricke-Glosser dosimeter using chamber no. 178 for unfiltered radiation. The dosimeter had been calibrated at the factory just previous to its use.

NUTRIENT SOLUTION.—The nutrient solution was solution B as used in my investigations on the germination of orchid seed (11), except that it was necessary to adjust the solution to a pH 5.7 to 6. This was done by using $K_2HPO_4$ instead of the monobasic potassium phosphate. No attention was paid to the addition of manganese, boron, copper, or zinc since these were present as impurities in the chemicals used. In certain of the experiments glucose or sucrose was added to the extent of 2 per cent.

This nutrient medium was heated in an autoclave at 10 pounds' pressure until the agar was "dissolved," and then approximately 30 cc. of the culture medium added to large culture tubes (21 × 2.5 cm.). The tubes were provided with cotton stoppers and autoclaved for 15 minutes at 15 pounds' pressure, after which they were placed in an inclined position to slope the agar medium.

STERILIZATION OF SPORES.—Following irradiation, the spores were placed in small test tubes 10 × 1 cm. and the tubes nearly filled with the filtered solution of calcium hypochlorite. The tube was closed by means of the forefinger and shaken until each spore was wetted. A 10–15 minute treatment was sufficient to free the spores of microorganisms. This is essentially the method developed by

WILSON (31), except that only 10 gm. of calcium hypochlorite was used to 125 cc. distilled water.

Previous experience had indicated that fern spores could be treated for considerable time with the calcium hypochlorite without exhibiting evidence of injury. Nevertheless it was thought desirable to determine definitely the effect of the duration of treatment. The usual nutrient solution was used, to which was added 2 per cent glucose to facilitate growth of any microorganism that might be present. All treatments were in triplicate. The exposures to calcium hypochlorite varied from 10 to 50 minutes. The results on germination and growth are shown in table 1.

TABLE 1

INFLUENCE OF TIME OF TREATMENT WITH HYPOCHLORITE ON GERMINATION AND GROWTH; DURATION OF EXPERIMENT, MARCH 30 TO APRIL 14

| TIME OF TREATMENT (MINUTES) | PERCENTAGE GERMINATION | AVERAGE NO. OF CELLS PRODUCED |
|---|---|---|
| 10 | 91 | 2.8 |
| 20 | 91 | 2.6 |
| 30 | 90 | 2.5 |
| 40 | 89 | 2.6 |
| 50 | 90 | 2.7 |

It is apparent from table 1 that the germination and growth are uninfluenced by the calcium hypochlorite, at least up to a 50-minute treatment. These cultures were permitted to develop to the large prothallial stage. Observations at this later period revealed no differences between cultures of the various treatments.

SOWING SPORES.—For sowing the spores in the tube, a looped platinum needle such as is used in bacteriological work was employed. The spores float to the surface of the hypochlorite solution and it is necessary only to dip the loop of the needle in the mass of floating material. By practice, an approximately equivalent amount may be removed each time and spread over the surface of the agar slope. Approximately 4000 spores were sown in each tube.

CULTURAL CONDITIONS.—All cultures were placed under a cloth shade in the greenhouse. Two layers of cheesecloth were used on

the top of the shade chamber, which was 3 feet in height, and a single layer of cheesecloth was used for the sides. The maximum light intensity was 400 foot-candles. The temperature ranged from 65° to 85° F., the lower temperature prevailing at night. In the daytime effort was made to keep the temperature close to 75° F.

ISOLATION OF PROTHALLIA.—Ten to 12 days after sowing, the spore coat was ruptured and the first rhizoid made its appearance. Observations on germination and growth were begun on the twelfth day and made at intervals thereafter. At the very outset of the experiments some chloroplast differences were noted. Attempts to isolate protonema with abnormal plastids were made but such transplants failed to survive. Cultures nos. 18 and 188, however, were isolated when in young prothallial stages of thirty and fifty cells respectively. These will be described subsequently. Most of the prothallia exhibiting definite plastid abnormalities were isolated when in a more advanced stage.

All prothallia for examination were mounted in water on a microscope slide and gently teased apart with dissecting needles under a dissecting binocular microscope, using a magnification of 30×. Some of the plastid changes could be observed with this magnification although initially some of the prothallia with plastid abnormalities were missed. Later, prothallia were examined under a magnification of 90×. To isolate abnormal or other desired prothallia, use was made of flexible glass needles made by drawing out glass rods. These needles were about 20 $\mu$ in thickness and could be inserted under the coverglass and brought to the desired prothallium, which was pushed to the edge of the slide. It was then placed on a separate slide, and using the same glass needles the prothallium was freed of any adherent spores, protonema, or other prothallia. This prothallium was then picked up by means of a fine steel needle and transferred to a culture tube containing the nutrient medium.

REPRODUCTION OF PROTHALLIA.—After isolation, the prothallium of course no longer remained free of microorganisms. The tubes to which such isolated prothallia were transferred contained only the nutrient salts and no sugar. It was not therefore a medium favorable to the growth of microorganisms. Nevertheless some of the prothallia failed to survive the transfer. Some were bruised in the

handling and became covered later with a bacterial or fungus growth. This was especially the case when young prothallia (30- or 40-celled stage) were transferred. Fortunately prothallia representative of all types survived and reproduced vegetatively. It was observed early in the work that, under the greenhouse conditions provided, antheridia are not formed. Sexual reproduction of sporophytes is thus not possible and the prothallium continues to grow and reproduce new prothallia. At first protonemal threads arise from the prothallium, developing quickly into prothallia. Ultimately the entire agar surface may be covered with prothallia, which may be heaped up ten or more in depth. The quantity of prothallia obtainable is limited then only by the number of tubes or flasks provided.

**Experimentation**

Before considering plastid changes, the results of the effects of X rays on germination and growth will be presented. This is particularly desirable since there appears to be close correlation between plastid changes and the effect of X rays on germination and growth. For germination studies, samples were taken from representative cultures and observations made on at least 400 spores. The first observations were made at the end of 12 or 14 days, when the first evidences of germination may be noted. Subsequent observations were made at intervals until prothallia had reached an advanced stage. In the controls there was no appreciable increase in the percentage of germination after 20 days, but in those cultures sown with spores treated at 25,000 r units or more some germination occurred after this time, although no very significant increases were noted. Germination was considered to have occurred when the spore coat became ruptured. In those cultures with high intensities of treatment, 25,000 r units or more, many of the spore cells merely enlarged, their plastids developed, and such single-celled individuals would continue to live and enlarge, remaining in a healthy state. Observations on some of these single-celled individuals were made over a period of 18 months. How much longer they would have survived cannot be stated.

In addition to the data on germination, growth measurements were made at approximately the same intervals. These measure-

ments consisted merely in counting the number of cells of the protonema or young prothallia.

The data on germination are shown in figure 1. They are relative values and represent the results of three series of experiments, series 9, 10, and 11. In these the control is given a relative germination value of 100. The actual values for the controls of the three series were respectively 81, 75, and 96 per cent. The data were taken when germination of the control was practically complete.

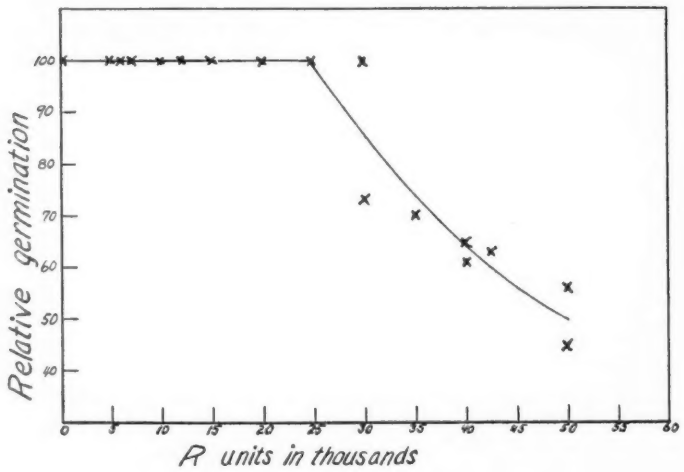



FIG. 1

In experiment 10, the treatments of spores were 0, 7000, 10,000, 12,000, 15,000, and 20,000 r units. Germination varied from 73 to 78 per cent, and to all these a relative germination value of 100 was assigned. This is in agreement with other experiments. In experiment no. 9 a series of treatments from 20,000 to 50,000 r units was provided by increments of 5000 r units. The percentage germination for the control was 81 and for the 30,000 r treatment 79. In experiment 11, however, the percentage germination in the culture with 30,000 r unit treatment was 77 against 98 for the control. The decrease in germination first occurs therefore at about 30,000 r units.

Under favorable conditions spore germination has occurred before the twelfth day and one cell division has occurred, and within a month a 12-celled protonema may be observed. Figure 2 gives the relative numbers of cells for the treatments 0 to 50,000 r units. These are taken from experiments 9 and 10 since the observations in these experiments were made at the end of approximately one month. The data from 20,000 to 50,000 r units are not only rela-

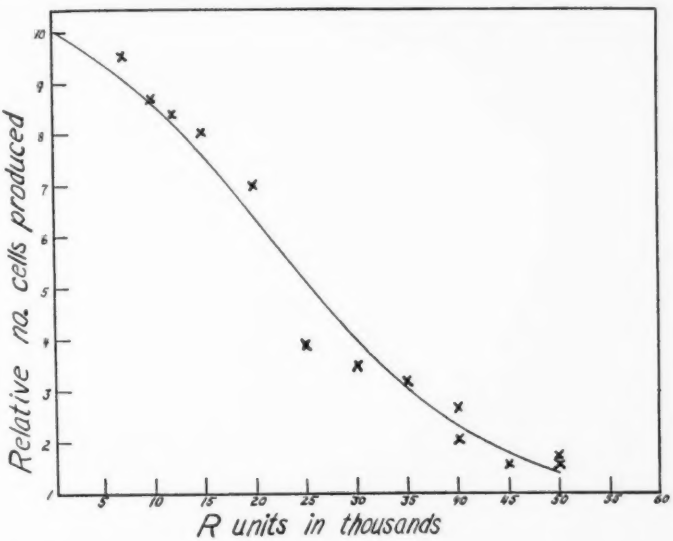



FIG. 2

tive but actual figures, since the control had made a growth of ten cells. The data for experiment 9 are given also in table 2. The data on growth reveal a progressive decrease with increasing intensities of irradiation. As shown by table 2 and also in figure 2, a large percentage of the plants show only a few cells. Nevertheless at these higher intensities some prothallia develop and attain a large size. It was among these that the isolations were made.

In experiments 9 and 11 a total of seventy-two cultures was provided. From twenty-five of these, fifty-one prothallia with abnormal plastids were isolated, the greatest number being obtained from ex-

periment 11 and particularly from series 11B. It is probable that in experiment 9 many prothallia with abnormal plastids were inadvertently missed. No abnormal plastids were observed in cultures treated with less than 30,000 r units. As indicative of the maximum frequency of these abnormal prothallia, consideration may be given to series 11B. In this series with sixteen cultures a total of approximately 64,000 spores were sown. From these approximately 12,672 prothallia developed from which thirty with abnormal plastids were

TABLE 2

EFFECT OF X RAYS ON GERMINATION AND GROWTH, EXPERIMENT NO. 9; DURATION OF EXPERIMENT, 30 DAYS

| IRRADIATION | PERCENTAGE GERMINATION | AVERAGE NO. OF CELLS FORMED | PERCENTAGE 2-CELLED |
|---|---|---|---|
| Control | 81 | 10.17 | ..... |
| 20,000 r units | 83 | 7.01 | 31 |
| 25,000 | 80 | 3.95 | 41 |
| 30,000 | 79 | 4.13 | 42* |
| 35,000 | 57 | 3.19 | 48* |
| 40,000 | 53 | 2.08 | 49* |
| 45,000 | 51 | 1.62 | 84* |
| 50,000 | 37 | 1.64 | 83* |

* Or less than 2-celled.

isolated. The ratio is therefore 1 to 422, or on the basis of total number of spores, 3 to 6400. In series 11C the ratio is 1 abnormal prothallium to 2050 normal, and in series 11D the ratio is 1 to 242. In series 11D the total number of prothallia produced was only 2176 but the number of spores sown in the sixteen tubes was again 64,000. When the frequency is based on the number of spores sown, the ratio of abnormal plastid forms becomes very low. In series 11D this ratio is about 1 to 7000. Data on isolations of prothallia with abnormal plastids are given in table 3.

These isolations of prothallia with abnormal plastids (table 3) were made in the latter part of 1932. Since that time they have been multiplied by vegetative reproduction. They have been grown on the usual culture medium in tubes, in liquid culture media, on soil, and on osmunda peat. Irrespective of the culture medium used, the characteristics of the plastids for each type have been main-

tained except for those slight variations which are associated with nutritional conditions. Except for the prothallia with grouped plastids, there is little difference among the prothallia having the different plastid types. These abnormal plastids have persisted then in the gametophyte for more than 7 years, and it may be concluded that the plastid change in this gametophyte generation is permanent and will persist as long as prothallia are reproduced vegetatively.

TABLE 3

NUMBER OF PROTHALLIA SHOWING ABNORMAL PLASTIDS ISOLATED FROM THE DIFFERENT EXPERIMENTS

| SERIES NO. | R UNITS | NO. OF CULTURES | NO. OF ISOLATIONS | PERCENTAGE OF SPORES WHICH PRODUCED PROTHALLIA |
|---|---|---|---|---|
| 11B | 30,000 | 16 | 30 | 19.8 |
| 11C | 40,000 | 16 | 4 | 12.9 |
| 11D | 50,000 | 16 | 9 | 3.4 |
| 9E | 35,000 | 6 | 1 | 25 |
| 9F | 40,000 | 6 | 5 | 25 |
| 9G | 45,000 | 6 | 1 | 8 |
| 9H | 50,000 | 6 | 1 | 6 |

In order to simplify the presentation of data, a classification of the prothallia based on the plastids is presented in table 4. Including the normal type, eleven groups have been established. Within a single group there may be constant differences and therefore distinctive types, but the general similarity justifies the grouping and simplifies the discussion. Consideration has been given to the plastids in cells of all ages, but in the main the classification is based on the plastids in cells of intermediate age. In the very young cells the plastids are generally small and undeveloped; in the very old cells they may be extraordinarily modified. The distinctive characteristics of each are shown in the accompanying illustrations.

TYPE A, NORMAL PLASTIDS.—In prothallia produced from spores not irradiated, and likewise in nearly all prothallia produced from irradiated spores, the plastids are characteristically uniform in size and shape. They may be small, and platelike or discoid, with a thickness of about 1.5 $\mu$. In surface view the plastids when crowded

TABLE 4

CLASSIFICATION OF PROTHALLIA BASED ON PLASTID

| TYPE | DESCRIPTION OF PLASTIDS | CULTURE NO. | ORIGINAL EXPERIMENT | IRRADIATION (R UNITS) |
|---|---|---|---|---|
| A | Normal; small, roundish, or angular plates, uniform in shape | ..... | ..... | None |
| B | Grouped; collected in mass about nucleus | 96 | 9F3 | 40,000 |
| | | 121 | 11D5 | ..... |
| | | 187 | 11B8 | 30,000 |
| | | 188 | 11B8 | 30,000 |
| | | 195 | 11B5 | 30,000 |
| | | 272 | 11B6 | 30,000 |
| C | Giant; amoeboid forms; frequently vacuolate | 18 | 11C9 | 40,000 |
| | | 198 | 11B4* | 30,000 |
| D | Large thin plates; incomplete divisions or fusion with plastids stringlike, branched; others irregular; some vacuolate | 91 | 11C16 | 40,000 |
| | | 291 | 11B6* | 30,000 |
| E | Very large plates; in older cells slightly more irregular | 262 | 11B7* | 30,000 |
| F | Large thin plates appearing as mosaic; in older cells very irregular with fusions | 263 | 11B7 | 30,000 |
| G | Large thin plates, irregular outline, incomplete division or fusions; extraordinary vacuolations | 185 | 9F4 | 35,000 |
| H | Large plastids; square, rectangular, or rounded plates | 79 | 11B10 | 30,000 |
| | | 85 | ..... | ..... |
| | | 206 | 11B9 | 30,000 |
| | | 231 | ..... | 30,000 |
| | | 233 | 11B4 | 30,000 |
| | | 249 | 11D9 | 50,000 |
| | | 250 | 11D9 | 50,000 |
| | | 255 | 11D9 | 50,000 |
| | | 266 | ..... | ..... |
| | | 267 | ..... | ..... |
| | | 282 | 11B6 | 30,000 |
| | | 296 | 11B6 | 30,000 |
| I | Thin plates, variable in size, not large; surface appears rough owing to many starch grains | 148 | ..... | ..... |
| J | Small plastids; roundish to angular, others irregular, some vacuolate; relatively large starch grains; tending to group | 235 | ..... | ..... |
| K | In young cells plastids small, rounded; in older cells free of starch; spherical, with chlorophyll in bands or threads; vacuolate | 205 | ..... | ..... |

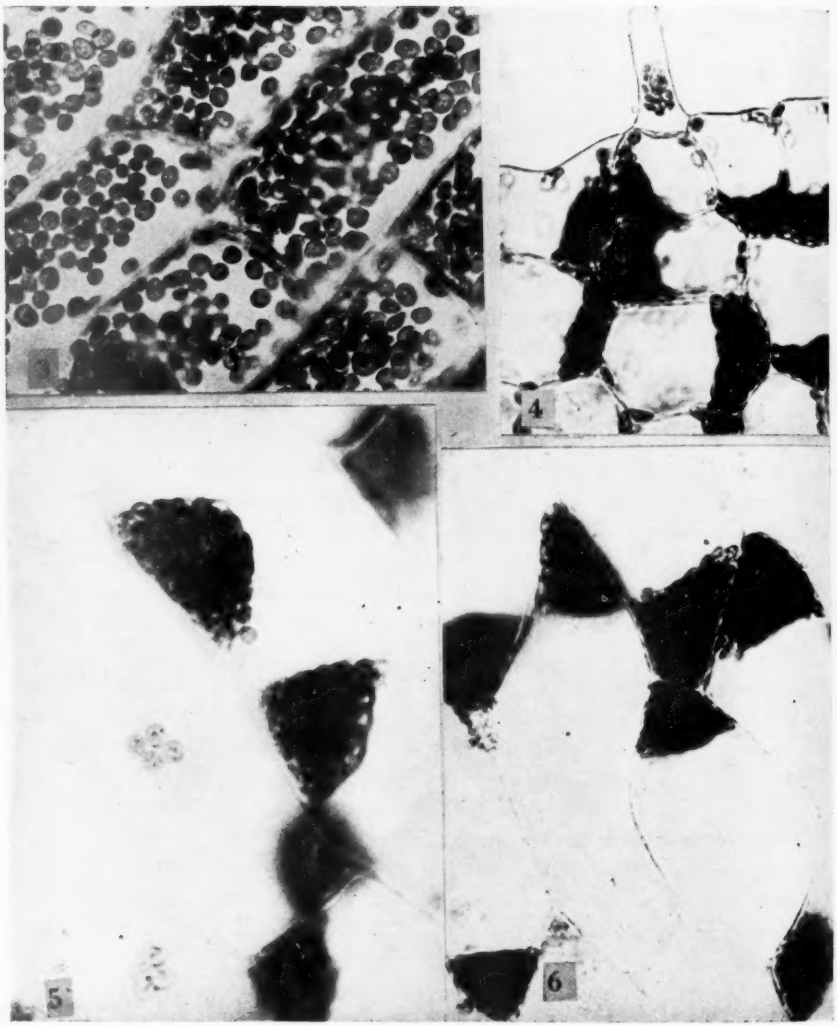
* Grown with 2% sucrose.

are angular, being squarish or rectangular to polyhedric; when less crowded they may be rounded in outline. In surface view they vary in size from $3 \times 4.5\ \mu$ to $6 \times 9\ \mu$. These values hold for plastids in healthy cells; in poorly nourished cells they may be appreciably smaller. Another characteristic is the large number of plastids per cell. When the plastids are uniformly distributed, 125 or more may be noted in either the upper or the lower periphery of the cell (fig. 3).

TYPE B, GROUPED PLASTIDS.—In the prothallia of *Polypodium aureum* and in plants in general the chloroplasts are distributed in the peripheral cytoplasm. Preceding cell division there may be a clustering of the plastids about the nucleus, but this grouping does not persist. In epidermal cells leucoplasts may be clustered about the nucleus, as in *Zebrina pendula*, and in *Elodea* under high light intensity such a clustering may occur. In the prothallia of group B the plastids are massed about the nucleus, generally near an end wall. Only occasionally is a plastid or a group of three or four found isolated in the peripheral cytoplasm (figs. 4–6). In these prothallia the nucleus of a cell is not visible and its position is made manifest only when light of high intensity is used. Under such conditions its position is made evident by a light spot within the mass of plastids. The extent of the grouping varies within the members of this group. Thus in cultures nos. 96, 272, and 195 it is rare to find an isolated plastid. In nos. 187 and 188 small groups of plastids may be isolated in the peripheral cytoplasm. It is difficult to measure the plastids grouped about the nucleus, since many show only an edge view while others appear to be distorted in shape. Those that are isolated are roundish plates. In no. 96 the diameter ranges from 4.5 to 7.5 $\mu$, in no. 121 from 3 to 7.5 $\mu$, in nos. 87 and 88 from 4.5 to 7.5 $\mu$, and in nos. 195 and 272 from 4.5 to 6 $\mu$. In size these plastids are like the normal.

These prothallia are thin-walled and are bluish green in color, although no. 272 is slightly more greenish than others of this group. The prothallia are easily distinguished macroscopically from all other types because of their unusual color and more delicate structure. They also appear to grow more slowly.

Light intensity is not a factor of importance in this grouping.




FIGS. 3–6.*—Fig. 3, type A: Typical normal plastids in prothallia of *Polypodium aureum;* note small size and large number in surface view. Figs. 4–6, type B: Grouped plastids densely packed about nucleus (fig. 4, culture 121; fig. 5, culture 188; fig. 6, culture 96); in figs. 4 and 5 a few plastids may be noted isolated in peripheral cytoplasm.

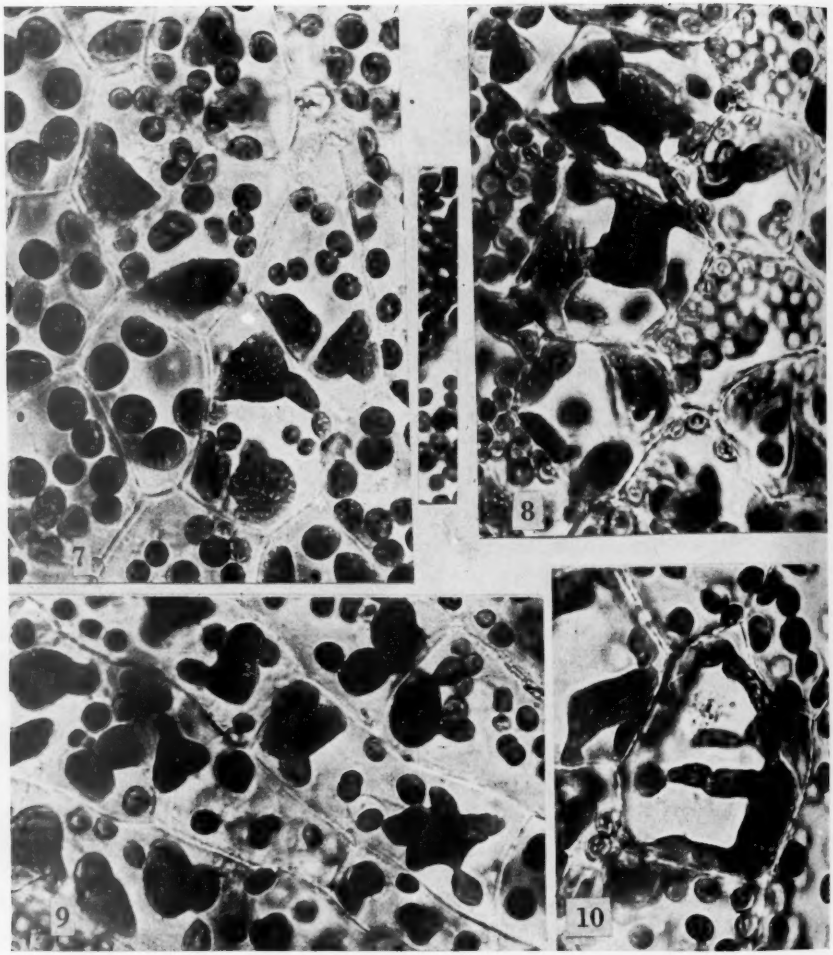
* Magnification in all figures is 500.

Cultures kept for 5 months under a laboratory bench, where the light intensity during the daytime was only 5 foot-candles, still exhibited plastids grouped about the nucleus. In the greenhouse, in cultures maintained under conditions where light intensity reached values of 2000 foot-candles, the grouping persisted.

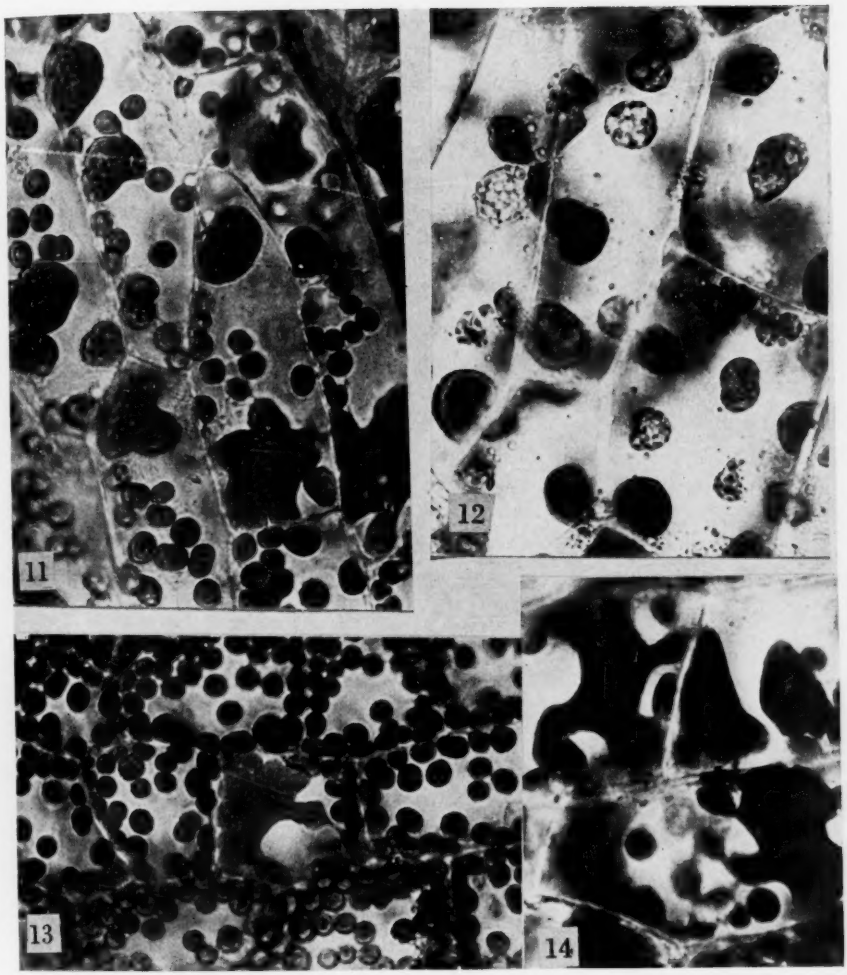
TYPE C, GIANT PLASTIDS AND AMOEBOID FORMS.—Only two prothallia of this type were isolated, nos. 18 and 198. The group is characterized by very large and irregularly shaped plastids located mostly in a layer of five–ten cells bordering the periphery of the prothallium. Because of the shape of these plastids they are termed amoeboid. They may be present in any cell of the prothallium except the apical and those younger cells bordering the notch. In these young cells the plastids are circular in outline and only 2–3 $\mu$ in diameter. There are about ten of these small plastids in each of the very young cells. In the cells toward the base of the prothallium the plastids are generally circular plates, varying in healthy prothallia from 7.5 to 15 $\mu$ in diameter. Even in these cells an amoeboid plastid may be found. The striking features of these prothallia are the amoeboid plastids found in the cells of intermediate age. Usually these pastids are more common on the lower surface of the cell than on the upper (figs. 7–11).

The typically amoeboid plastids vary greatly in size and shape and assume grotesque forms. The evidence appears to show that they are formed by the union of smaller plastids, and then by a budding process give rise again to the smaller plastids (figs. 7, 11, 14). Because of their curious outline it is difficult to measure them. In some cells a single large rectangular plastid may be observed with dimensions of 50 $\times$ 70 $\mu$. Long bandlike plastids have been observed 150 $\times$ 6 $\mu$. L-shaped plastids occur with the long arm 60 $\times$ 23 $\mu$ and the short arm 45 $\times$ 15 $\mu$. Of the irregularly shaped plastids the maximum dimension may vary from 20 to 60 $\mu$. The frequency of the amoeboid plastids seems to be correlated positively with the vigor of growth of the prothallium.

Culture no. 198 is similar to no. 18 but appears to differ from it in two respects. The plastids tend to be more commonly circular in outline, with diameters of 6–15 $\mu$. The amoeboid forms are less frequent in no. 198 than in no. 18, but more plastids are "vacuo-




FIGS. 7–10.—Type C: Amoeboid type plastids, culture 18, illustrating striking size and varied forms. Figs. 7, 8, certain cells shown possessing only circular form of plastid. Fig. 9, yeastlike budding of plastids shown clearly. Fig. 10, very large and irregular plastid. Insert, normal plastids.

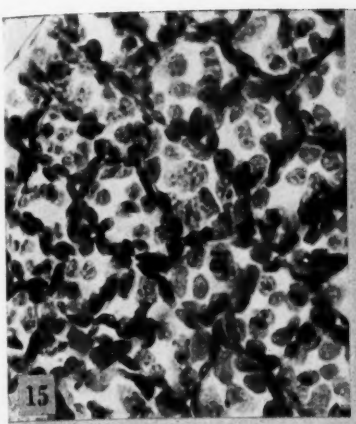


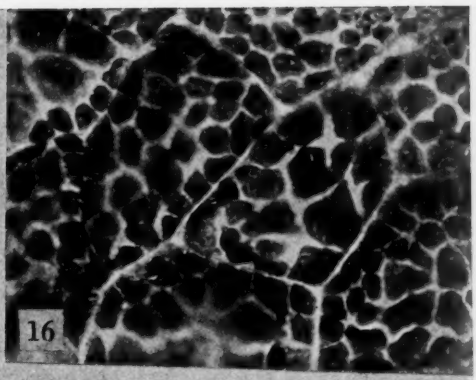

FIGS. 11–14.—Fig. 11 (type C, culture 18), showing yeastlike budding of plastids. Figs. 12–14, type C: Amoeboid plastids, culture 198. Fig. 13, inner cells of prothallium showing predominance of circular plastids; fig. 14, peripheral cells showing large amoeboid types; fig. 12, older cells showing vacuolate type plastids. Some plastids are swollen and chlorophyll is irregularly disposed, having the appearance of a foamlike structure owing to unusual disposition of chlorophyll.

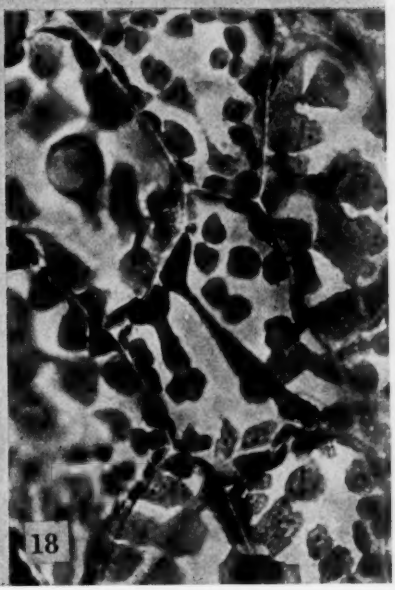
lated" in no. 198 than in no. 18 (fig. 12). Some of these vacuolated plastids present the appearance of rings with jewel settings. Others in outline form present the appearance of the figure 8. In reality the latter consists of two spheres and the ringlike plastids are nearly spherical in shape; in both cases the chlorophyll is not uniformly distributed but occurs in bands. This will be considered subsequently when discussing the plastids of group J.

TYPE D, LARGE THIN PLATES, IRREGULAR IN SHAPE, FUSIONS.—Cultures nos. 91 and 291 have been placed in this group because in the very young and medium aged cells the plastids are much alike. In the young cells the plastids are angular plates of variable sizes; in the medium aged and older cells large platelike plastids prevail and are variable in size. These have dimensions of $30 \times 3\ \mu$, $9 \times 9\ \mu$, $6 \times 3\ \mu$, $15 \times 16\ \mu$, $12 \times 9\ \mu$, $15 \times 12\ \mu$, and some with maximum dimension of $60\ \mu$ (figs. 15–18, 19–22). In cells of medium age, plastid masses may be held together by thin green threads. In the older cells the plastids seem to have fused, especially in no. 291 (figs. 21, 22). Vacuolated plastids are common (figs. 18, 22). This condition is so pronounced in the older cells that some plastids appear to have a frothlike structure (fig. 22). The starch grains in all plastids are large.

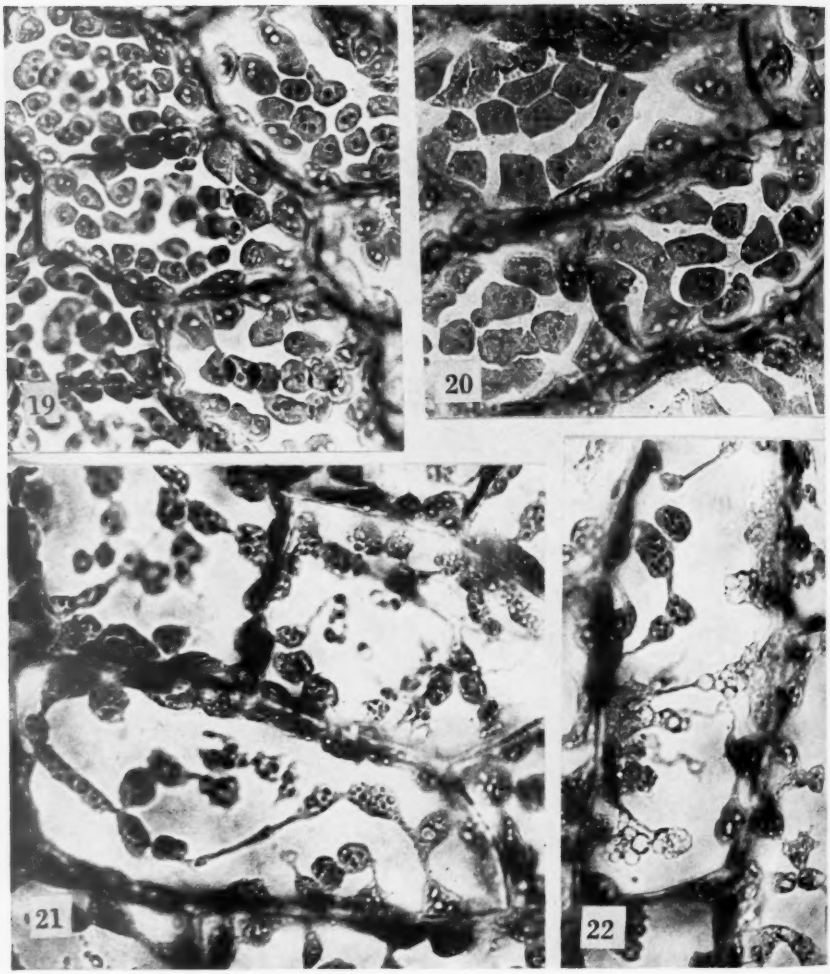
TYPE E, VERY LARGE THIN PLATES; CULTURE NO. 262.—Only one prothallium of this type was observed and isolated. It survived and reproduced other prothallia in abundance. It is a vigorous grower. In the very young cells adjacent to the apical cell the plastids tend to be rodlike, expanding in size to small plates in older cells. In fully formed cells of intermediate age—and therefore in the greater part of the prothallium—the plastids are thin plates and attain extraordinary size. In outline they are generally angular and various sizes may occur in the same cell. The smaller plastids vary from $10 \times 8\ \mu$ to $20 \times 6\ \mu$. The large ones may be $60 \times 38\ \mu$, $20 \times 20\ \mu$, or $45 \times 22\ \mu$ (fig. 23). In the older cells (figs. 24, 25) the plastids are generally smaller, being strandlike with swollen ends, recurved, circular in outline, or bands. In all of them the starch grains are numerous and very prominent. These plastids, while possessing a large area, are usually thinner than are normal ones.






FIGS. 15–18.—Type D: Irregular thin plates, culture 91. Fig. 15, young cells near growing point; plastids irregularly shaped plates and relatively large for such cells. Figs. 16, 17, later stages in plastid development. Fusion appears to have occurred, yielding large plastids as in fig. 17. Fig. 18, irregularity of shape and vacuolation of plastid.



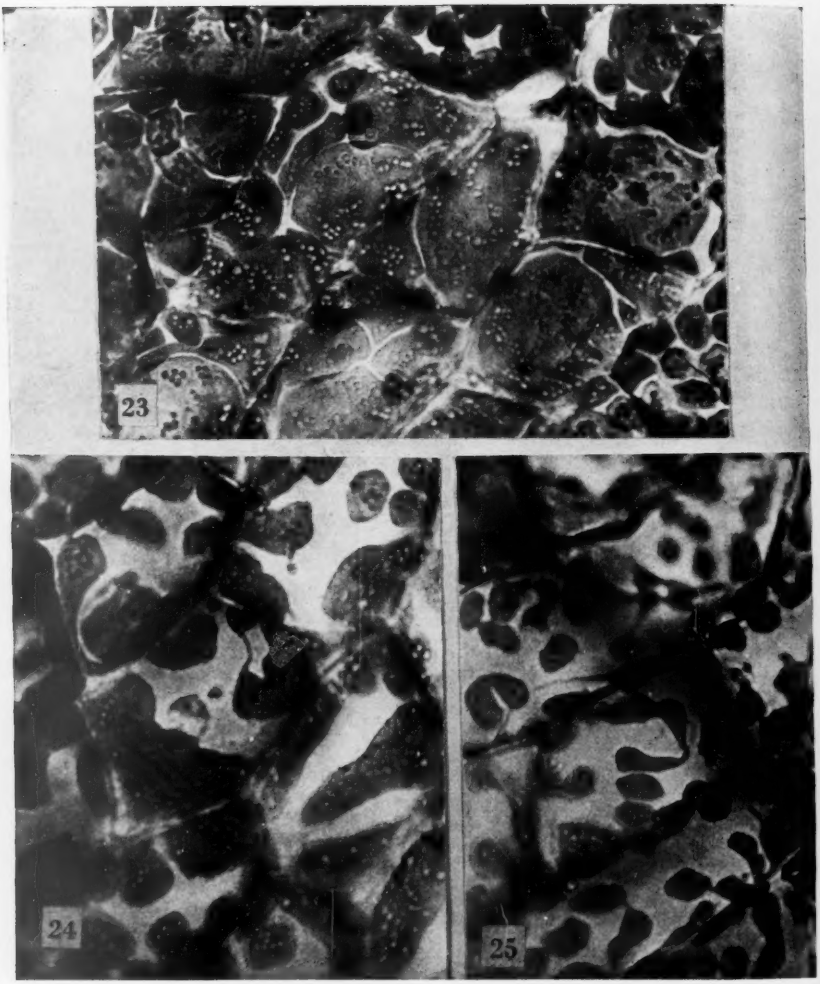

FIGS. 19–22.—Type D: Culture 291. Fig. 19, young cells. Fig. 20, older cells showing irregularly shaped plate plastids. Fig. 21, fusion of plastids. Fig. 22, vacuolization.

GROUP F, IRREGULAR THIN PLATES, FUSIONS; CULTURE NO. 263.—In the very small cells close to the apical cells the plastids are similar to those of no. 262, appearing as small rods or angular or circular plates. In cells slightly older many of the plastids appear to fail to separate, remaining connected by thin green strands (fig. 26). In the older cells the plastids, while platelike, show great irregularity in shape and size. In these older cells of healthy prothallia, this irregularity of outline is suggestive of the irregular splitting of a thin film of clay and may be likened to the appearance of a jigsaw puzzle (fig. 27). In the large mature cells the plastids become elongated masses of plastid material connected by long green strands (fig. 28). In many of these older cells fusions appear to have occurred, with most of the plastids interconnected by green strands (fig. 29) in which small starch grains may be noted.

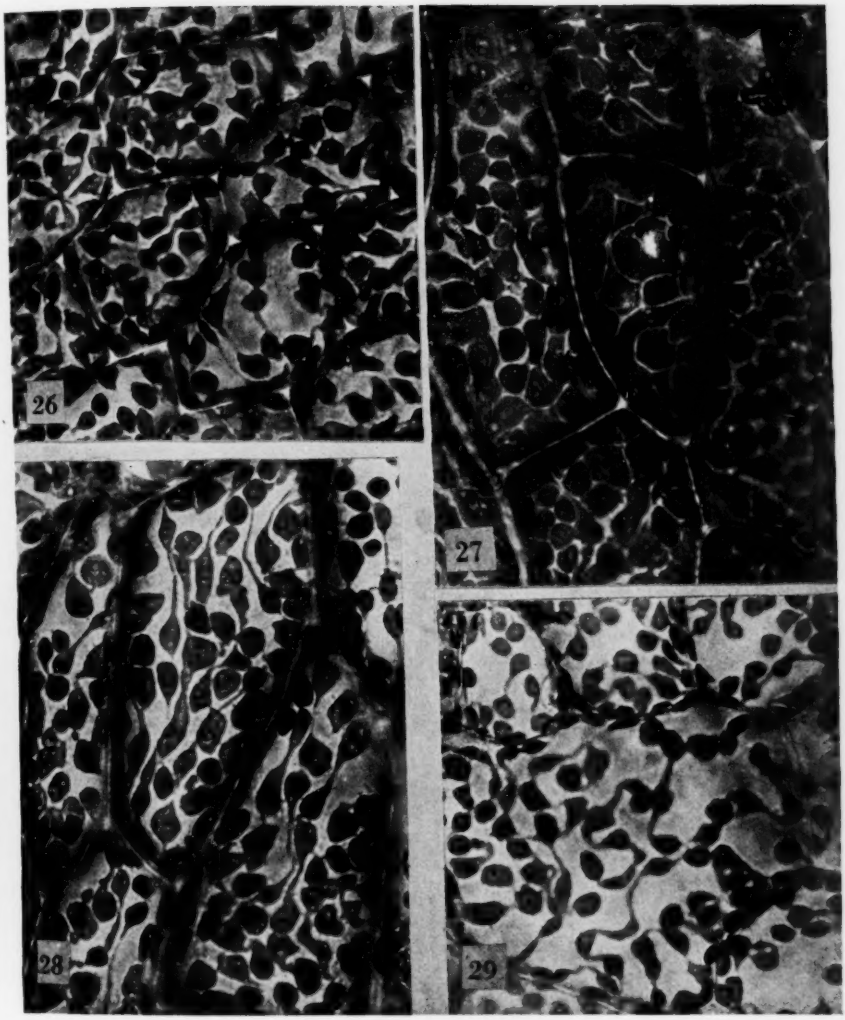
In general the plastids of no. 263 are smaller than those of no. 262 and more numerous per cell. Because of the irregularity of shape it is difficult to measure the plastid. In cells of intermediate age those plastids that can be easily measured show dimensions as follows: $9 \times 9\,\mu$, $7 \times 4\,\mu$, $5 \times 15\,\mu$, $21 \times 9\,\mu$, $25 \times 15\,\mu$, $33 \times 8\,\mu$, and $30 \times 12\,\mu$. The largest rectangular plastid measured was $90 \times 18\,\mu$.

TYPE G, THIN PLATES, VARIABLE IN SIZE, VACUOLATED; CULTURE NO. 185.—Only one prothallium of this type was isolated. In the apical and adjacent cells the plastids are minute, and angular or circular in outline. These quickly enlarge, so that in young cells platelike plastids appear with prominent starch grains (figs. 30, 32). In cells slightly older, larger plates are noted. These are irregular in outline and many of the plastids are vacuolated (fig. 31). Plastids with enlarged ends connected by thin green strands are common (fig. 33). In old cells irregular bandlike plastids occur which appear to be the result of fusion. In cells of intermediate age the rectangular plastids have dimensions of $6 \times 30\,\mu$, $6 \times 18\,\mu$, and $9 \times 18\,\mu$. Circular plates $15\,\mu$ and some only $7\,\mu$ in diameter may be observed.

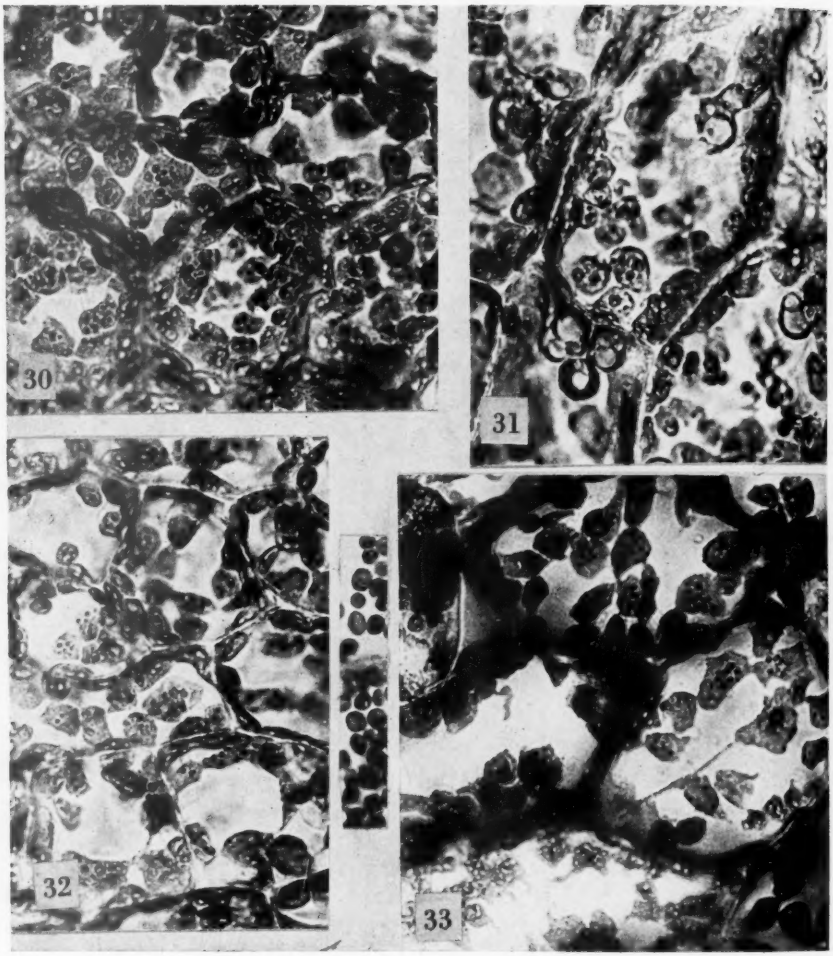
TYPE H, LARGE PLASTIDS.—This type of plastid change occurred with the greatest frequency. Altogether twenty-one prothallia exhibiting large plastids were isolated, and of these twelve survived. For the most part they are much like the normal ones in shape and




FIGS. 23–25.—Type E: Culture 262. Fig. 23, relatively young cells with very large plate plastids containing many starch grains, and smaller plastids interspersed. Fig. 24, shrinkage of plastids in older cell. Fig. 25, older cells; plastids relatively small plates and strandlike.




FIGS. 26–29.—Type F: Irregular plates and fusion, culture 263. Fig. 26, young cells near growing point; strandlike connections between plastids. Fig. 27, older cells; plastids forming pattern like jigsaw puzzle, characteristic of type F. Fig. 28, long green strands connecting enlarged portions of plastid. Fig. 29, fusion resulting in long meandering plastid.



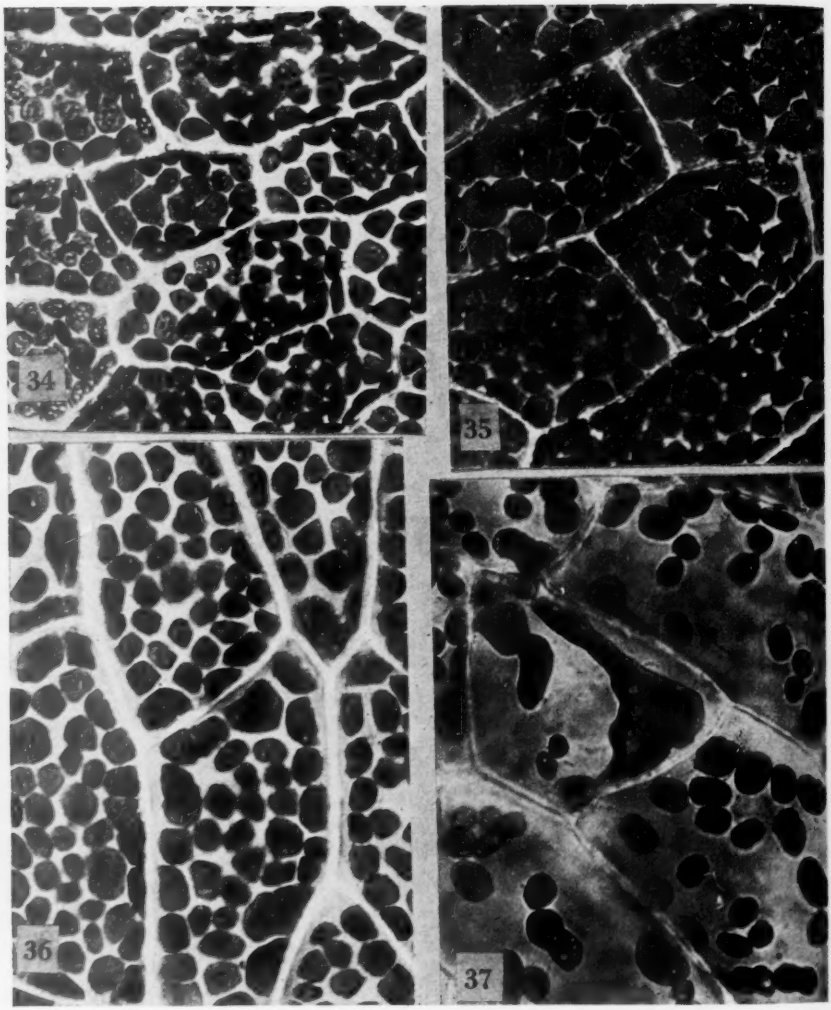

FIGS. 30–33.—Type G: Culture 185. Figs. 30, 32, young cells showing irregularity of size and shape of plastids. Fig. 31, older cells with larger plastids, some vacuolate. Fig. 33, medium aged cells with plastids possessing thin strands connecting swollen ends of plastid. Insert, normal plastids.

form but are very much larger, having at least twice as much area (figs. 34–37). Culture 296 exhibits at times unusually large plastids somewhat similar to the amoeboid type, but for the most part the plastids conform to the type of this group. In young cells (fig. 34) the plastids are angular plates 6–10 $\mu$ in maximum dimension. In older cells they are angular plates, although with fewer plastids to the cell circular plates may prevail. These tend to be variable in size. The circular plate types have diameters of 9–21 $\mu$; the rectangular plastids vary from 6 × 15 $\mu$ to 15 × 22 $\mu$. Some tend to become vacuolated, although this is not common. In general the number per cell is much less than in the normal. In the normal cell there may be 125 or more in a surface view, but only thirty to forty of the large plastids are present in the peripheral cytoplasm.

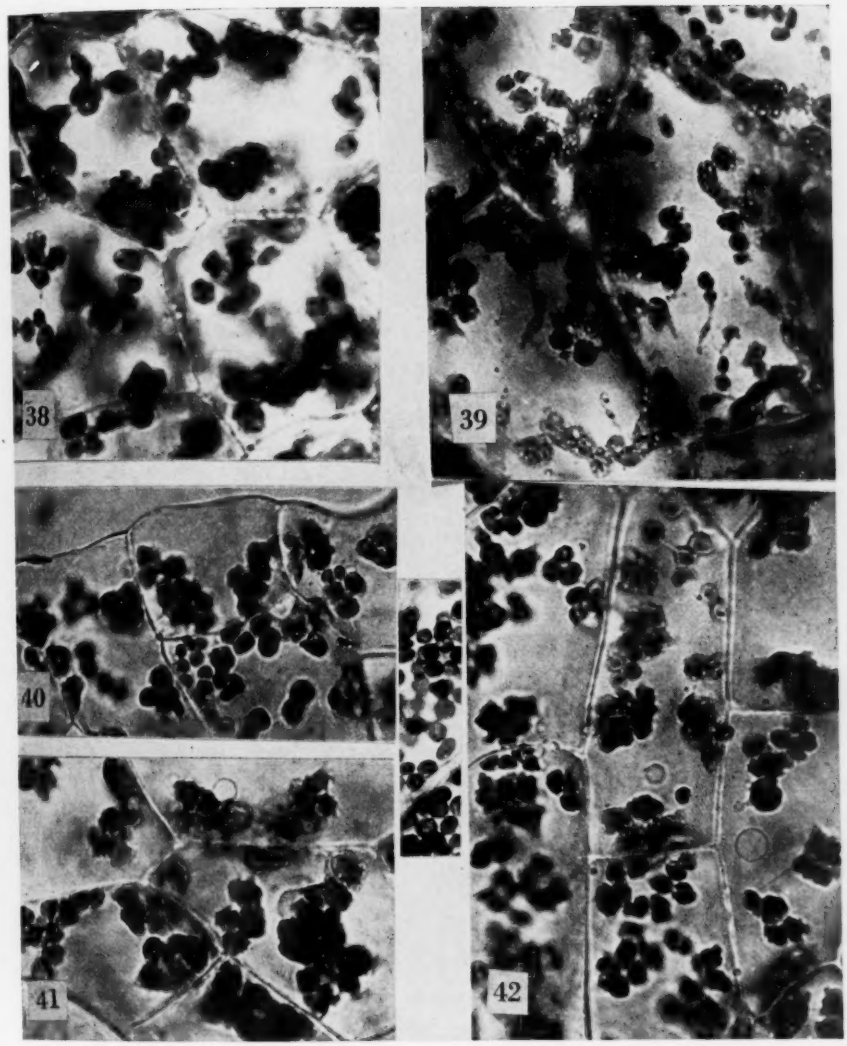
TYPE I, THIN PLATE GENERALLY SMALL, VARIABLE IN SIZE; CULTURE NO. 148.—Only one prothallium of this type was isolated. In the very young cells the plastids are minute plates, circular or angular in outline. In well developed cells they are thin plates with dimensions of from 3 × 4.5 $\mu$ to 7.5 × 7.5 $\mu$. This is about the size of the normal plastids but larger ones may also be noted, some having dimensions up to 15 × 18 $\mu$. Some are connected by thin green strands, while vacuolated plastids are not uncommon. The surface of the plastid appears rough because of the large starch grains (figs. 38, 39).

TYPE J, PLASTIDS SMALL, VARIABLE IN SHAPE, AND TENDING TO GROUP; VACUOLATED; CULTURE NO. 235.—These plastids resemble those of culture no. 148 of type I, but they are more uniform in size, smaller, and tend to group. The circular plate plastids may vary in size, diameters being from 4 to 10 $\mu$. The rectangular plastids have dimensions of from 4.5 × 6 $\mu$ to 6 × 7 $\mu$ (figs. 40–42). A few large ones may have dimensions of 4.5 × 12 $\mu$. Strandlike plastids are occasionally noted. Vacuolated plastids occur and in nearly every cell in the plane of the plastids a colorless ringlike structure may be observed. Such rings are variable in size (figs. 41, 42) and are probably plastids free of starch and chlorophyll which have assumed the vacuolated condition.

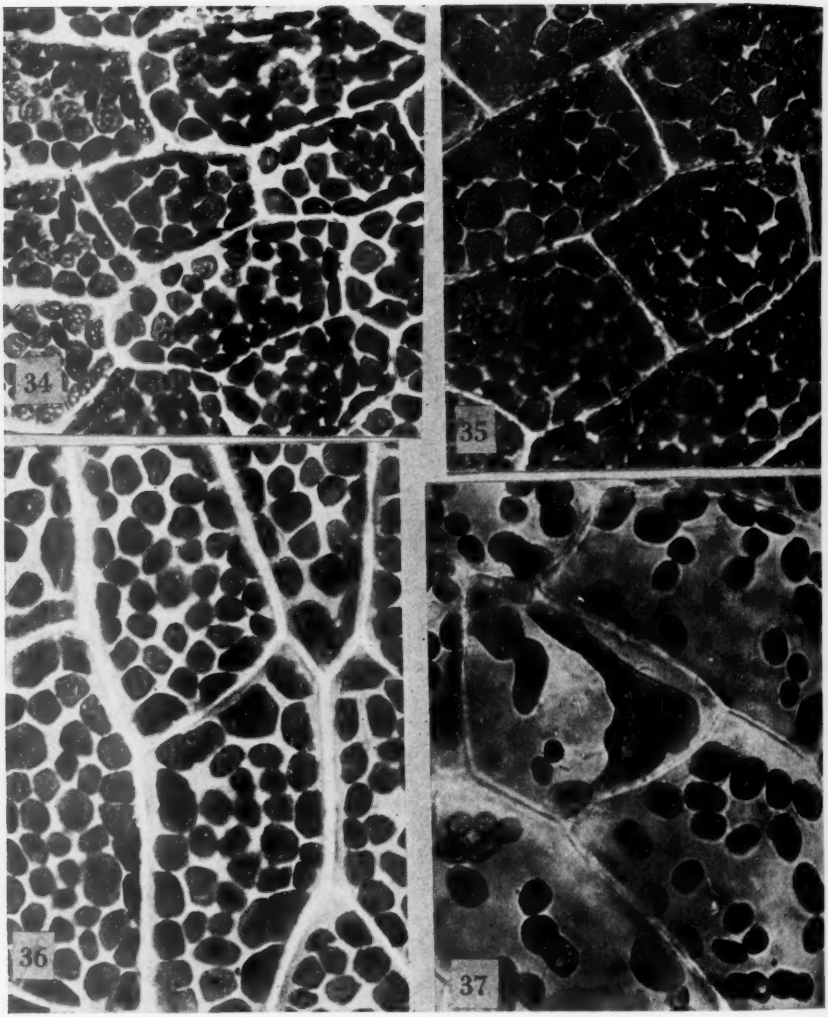
GROUP K, VACUOLATED PLASTIDS; CULTURE NO. 205.—Two prothallia of this type were isolated but only one survived. This is



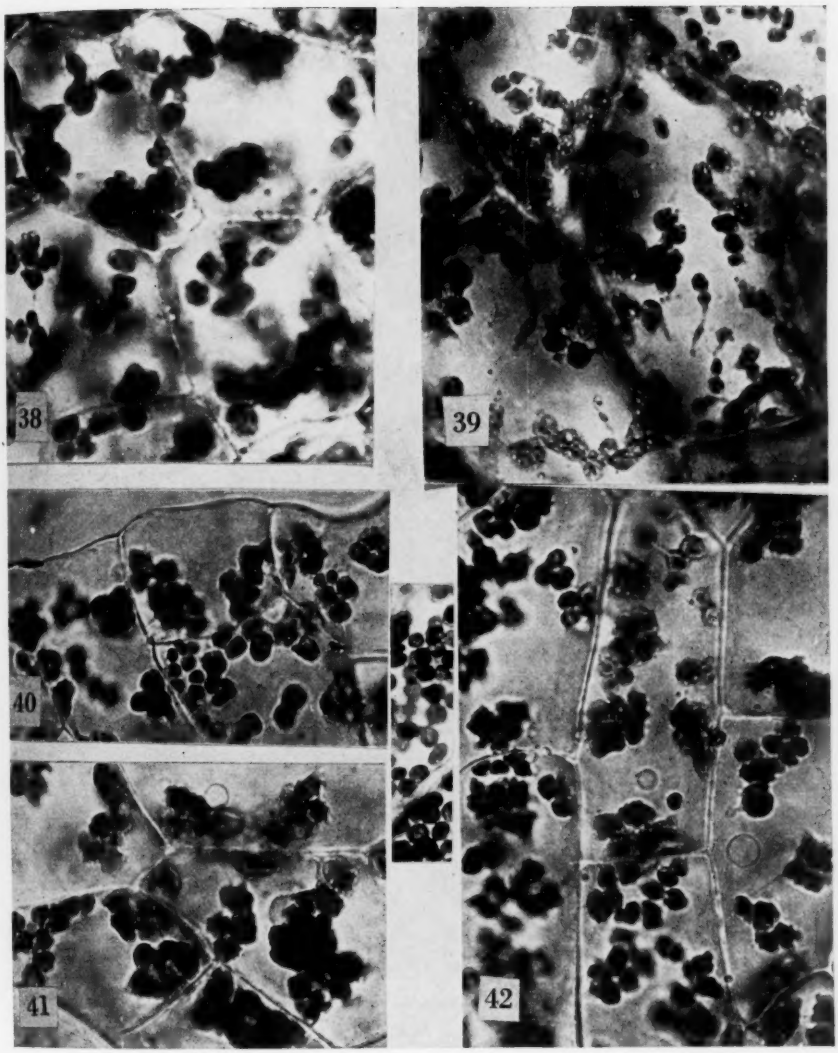

FIGS. 34–37.—Type H: Large plastids. Fig. 34, young cells near growing point of prothallium, culture 266; note small number of plastids and relatively large size. Fig. 35, slightly older cells of same prothallium. Fig. 36, mature cell of culture 266, showing characteristic large plastids. Fig. 37, unusual plastid found occasionally in culture 266.



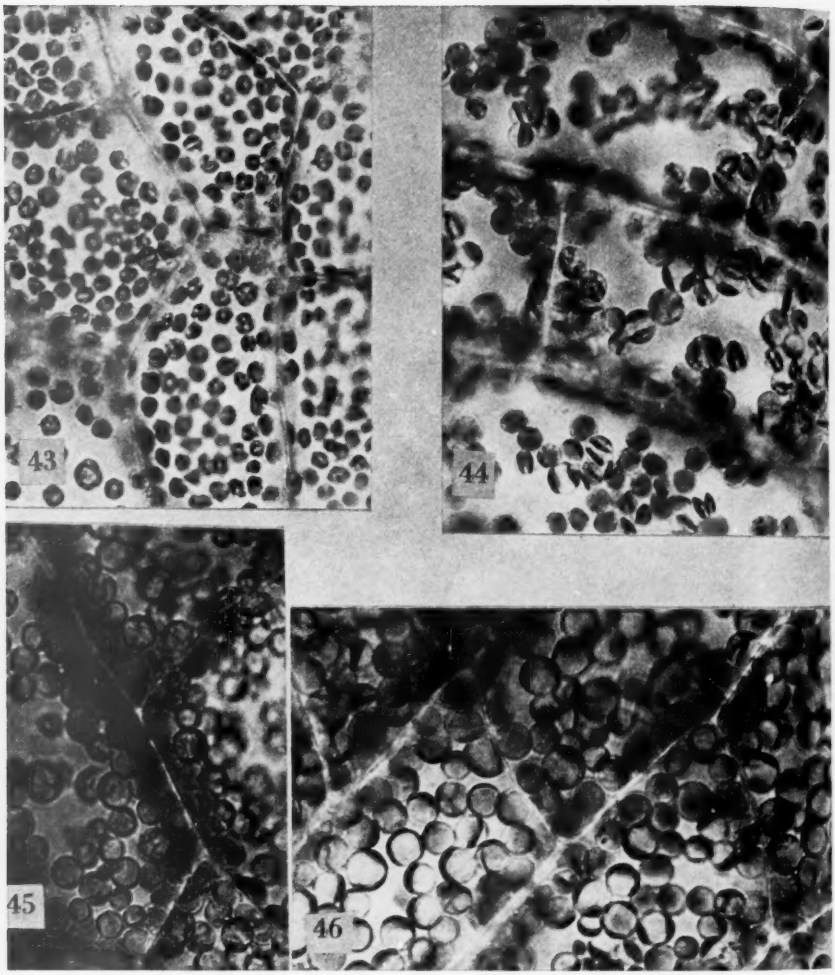

FIGS. 38–42.—Figs. 38, 39, type I: Culture 148. Fig. 38, plastids in young cells; fig. 30, old cells with plastids tending to coalesce. Figs. 40–42, type J: Culture 235. Fig. 40, young cells near growing point; figs. 41, 42, older cells showing grouping tendency; ringlike structures, probably vacuolate plastids, shown especially well in figs. 41 and 42. Insert, normal plastids.




FIGS. 34–37.—Type H: Large plastids. Fig. 34, young cells near growing point of prothallium, culture 266; note small number of plastids and relatively large size. Fig. 35, slightly older cells of same prothallium. Fig. 36, mature cell of culture 266, showing characteristic large plastids. Fig. 37, unusual plastid found occasionally in culture 266.




FIGS. 38–42.—Figs. 38, 39, type I: Culture 148. Fig. 38, plastids in young cells; fig. 39, old cells with plastids tending to coalesce. Figs. 40–42, type J: Culture 235. Fig. 40, young cells near growing point; figs. 41, 42, older cells showing grouping tendency; ringlike structures, probably vacuolate plastids, shown especially well in figs. 41 and 42. Insert, normal plastids.




FIGS. 43–46.—Type K: Culture 205, vacuolate type. Fig. 43, young cell showing typically small plastids with chlorophyll concentrated in small bands and threads. Fig. 44, slightly older cells with chlorophyll in band and threadlike arrangement. Starch entirely lacking in these plastids and in those of figs. 45 and 46. Fig. 45, old cells showing plastids with equatorial bands of chlorophyll, others with bands and irregular threadlike strands of chlorophyll. Fig. 46, mature cells; chlorophyll largely disposed in equatorial band.

probably the most extraordinary plastid of all. In young cells of healthy dark green prothallia the plastids appear almost normal, except that their appearance suggests a slightly more liquid consistency. Starch is present in small granules and is scant. In slightly older cells the plastids show an irregular distribution of chlorophyll, possessing islets free of chlorophyll (fig. 43). In slightly older cells the plastid is entirely free of starch and appears spherical, with the chlorophyll in wide bands and thinner interconnecting bands. In still others the chlorophyll appears as an equatorial band. Many such variations occur (figs. 44–46). This type grows extremely slowly and is difficult to maintain in culture.

In seeking an explanation of this unusual distribution of chlorophyll, consideration must be given to the osmotic properties of the plastid. PRIESTLEY and IRVING (**22**) pointed out that plastids of a species of *Selaginella* when extruded into distilled water showed marked swelling and finally rupture. This swelling was likewise observed by LIEBALDT (**17**). PRIESTLEY and IRVING considered the swelling to be due to colloidal imbibition. In a brief paper I (**13**) emphasized the osmotic properties of the plastid and pointed out the probable presence of a semipermeable membrane. This will be reported in detail subsequently. Suffice it to state here that these plastids exhibit certain characteristics of plastids extruded into distilled water. They are spherical in shape and apparently the plastid material has actually pulled away from the chlorophyll in expansion. When a cell containing these plastids is plasmolyzed, these abnormal plastids are changed back to a type approaching the normal.

## Discussion

It is not the purpose of this paper to consider primarily the physiological effects of X rays. The data on germination and growth, however, are the result of many measurements and warrant a few statements. In her summary of the effect of X rays on green plants, JOHNSON (**10**) properly points out that many investigators have used an inadequate number of plants or have had too few replications to warrant definite conclusions. As has been done by others, she points out that in general the effect of X rays is deleterious to plants. My data are in agreement with this viewpoint. The spores

of *Polypodium aureum* require a high dosage of X rays to influence the germination and growth. The curves on germination and dosage (figs. 1, 2) reveal that the percentage of germination is decreased only when the dosage is as high as 25,000 or 30,000 r units. The growth curve resembles the survival curve as reported by PACKARD (21) for *Drosophila*. There is no stimulation of growth as was reported by SHULL and MITCHELL (24) for certain higher plants. In other experiments I have used dosages of 500–50,000 r units and no stimulation was noted. It is true that SHULL and MITCHELL used filtrated radiation, but in view of PACKARD'S work it is doubtful whether stimulation would result from filtered radiation. That the growth rate of *Polypodium aureum* can be used as a biological dosimeter is suggested by the results obtained, but at best the gametophyte stage of this plant could be used only to measure high dosages.

An interesting observation was the frequent occurrence of spores which failed to divide but continued to enlarge until large spherical cells were produced with diameters of approximately 100 μ. These cells were well provided with chloroplasts and contained large reserves of fat, usually with one large centrally disposed globule of fat surrounded by smaller droplets. Some of these cells appeared to be healthy after 18 months. How long they would have lived cannot be stated.

Mention has been made previously of the fact that fifty-one prothallia with abnormal chloroplasts were isolated. These were obtained only from cultures irradiated at 30,000 or more r units. Seventy-two such cultures were provided, each with about 4000 spores, so that to obtain these prothallia approximately 300,000 spores were sown. The type with large plastids yielded the greatest number of isolations and was followed closely by the type with grouped plastids. Thus of the fifty-one prothallia isolated, twenty-one had large plastids (type H), fourteen were of the grouped type (type B), five of the vacuolate type (type K), two of the amoeboid type (type C), and four of type D. Only one prothallium was obtained of each of the other types.

It has been emphasized that these new plastid types have persisted in culture for more than 6 years and that each type maintains its characteristics, irrespective of cultural conditions. Since

no plastid changes were found in cultures irradiated below 30,000 r units, it is clear that the changes were caused by irradiation. In other investigations in this laboratory with the same fern it is probable that several million prothallia have been examined microscopically. No plastid changes were observed.

In investigations on the effect of X rays on green plants, chlorophyll variations have been noted (**18, 4**), suggesting that these might involve structural changes in the plastids. The only reference to such changes are those which I (**12**) reported in 1934 and which are amplified in this paper, and the plastid changes reported by HRUBÝ (**8**) in 1935. The work of HRUBÝ is particularly pertinent to my work. HRUBÝ, while associated with the late Dr. BROŽEK of Prague, exposed spores of *Equisetum arvense* to filtered radiation. Using an aluminum filter 0.5 mm. in thickness, amoeboid plastids of the type that I have described occurred in a high percentage of prothallia. With an exposure of spores to 2355 r units, 21.6 per cent of the prothallia possessed amoeboid plastids, while an exposure to 6800 r units resulted in amoeboid types being found in 46.5 per cent of the prothallia. No other types of plastids were reported. It will be noted that the frequency of prothallia exhibiting plastid abnormality is extraordinarily greater than in my work. This may be due to the fact that the spores of *Polypodium* are low in water content, while the spores of *Equisetum* appear to be in a much more active state physiologically and to have a higher water content. HRUBÝ makes no statement concerning the permanency of these plastid changes. It is probable that continued growth of the prothallia was not possible under the cultural methods he employed.

That cytoplasmic alterations may occur as a result of X ray treatment is evident from the results of various investigations, which have been summarized by GOODSPEED and UBER (**4**). Of particular significance here is the work of BIEBLE (**1**), who exposed *Bryum capillare* to α radiation. Agglutination and destruction of plastids followed. NADSON and ROCHLIN (**19**) state that the chondriosomes (probably proplastids) are more sensitive to X rays than is the nucleus. In their work, the chondriosomes of the epidermis of the onion scale after exposure to X rays swelled, became modified in shape, and fragmented.

In his monograph on the pathology of chloroplasts, KÜSTER (15) summarizes the work on plastid abnormalities. For the most part abnormalities were induced by mechanical or chemical means. Centrifuging, treatment with solutions of various alcohols, chloralhydrate, etc., resulted in various plastid modifications; but concerning the persistence of these changes no data are given. One of the more interesting cases was reported by KÜSTER (14) for *Bryopsis plumosa*. Plants cultivated in the laboratory for many months showed striking abnormalities in their plastids. Some filaments had normal plastids, others had long bandlike plastids, the greatest length being 280 $\mu$; other plastids were hook-shaped, amoeboid in form, or otherwise modified. In *Spirogyra* also plastid changes were noted.

These forms and those described by KÜSTER in his monograph—fusion, incomplete division, abnormal and unequal division, swelling, and so-called vacuolization—occur in the types that I have described. The significance of the work by KÜSTER in relation to my work is merely that the plastids are capable of markedly modifying their form under certain conditions. KÜSTER gives no evidence for the permanency of plastid changes reported by him.

Whether or not these changes are determined by chromosomal changes or are entirely independent of them cannot be stated at present. Cytological work on some of these forms is now in progress. That the plastids may differ independently of genic differences is apparent from the work of CORRENS and others. The following quotation from SINNOTT and DUNN (26) summarizes clearly the present status of the subject:

> Particularly important among these are certain traits in plants involving chloroplast development and constituting the so-called "albomaculatus" types of leaf variegation, in which the normal green tissue is irregularly spotted with patches of paler green or white, a type of variation intensively studied by CORRENS and others. These may be small or may include entire leaves or branches. This character occurs in a wide variety of plants, and its inheritance has been determined in more than 20 genera. Flowers in wholly green branches produce seed which grow into normal plants; flowers on variegated branches yield offspring which have variegated foliage, and flowers from branches wholly white give progeny without chlorophyll; but in every case the source of the pollen has no influence on the offspring. Inheritance is wholly maternal. Variegation seems clearly to be determined by agencies localized in the cyto-

plasm rather than in the chromosome. A satisfactory explanation of the mechanism of inheritance for such a trait is available, however, since variegation is evidently the result of differences in chloroplast development and since the primordia of these bodies, from which the plastids of the whole plant are ultimately derived, are present in the cytoplasm of the egg.

It is emphasized by SINNOTT and DUNN that while about forty such cases are known, at least several hundred cases are known where the plastids and chlorophyll modifications are controlled by genes. SIRKS (**27**) also summarizes a few of the special cases in plastid inheritance.

IMAI (**9**), recognizing that the plastid character may be controlled by genes, also emphasizes that the plastid may mutate. This mutation may be determined by a gene within the plastid; this he terms the plastogene. "Through plastogene mutation variant plastids appear among otherwise homogeneous plastids of a cell, variegation being exhibited when they propagate into [in] the cells." He states also that this automutation of plastids in meristematic cells is hindered and is less common in well nourished cells. The mutation to which he refers involves chlorophyll content and not form of plastid. At gametogenesis reverse mutation occurs and the zygote has only green plastids. At present it would seem that IMAI's data are inadequate to support the views he expresses.

In the introductory paragraph the question was raised whether or not the plastid changes are independent of any chromosomal alteration or aberration. No direct answer to this question is as yet possible. In my work great variation of plastids within a cell of a single type may occur. This is suggested evidence against genic control. In the case of the grouped type the behavior of the plastids in the sporophyte and succeeding gametophyte seems opposed to genic control. The plastids in this type are grouped about the nucleus, and in all prothallia derived from the original prothallium with grouped plastids this condition prevails. Various sporophytes have been obtained as a result of sexual reproduction, from prothallia of the grouped plastid type. Sporophytes have been obtained from cultures 121, 96, 187, and 188, and all have possessed the same plastid characteristics. At the very outset the young leaves and the stem have chloroplastids which are grouped about the nucleus, just

as in the gametophytic stage. Even the plastids in the guard cells of the stomates are grouped about the nucleus. This grouping occurs in all leaves produced during the first 2–3 years. The first leaves are several mm. to 2 cm. in length; by the third year leaves are produced which may be 6 inches in height. When the adult form of leaf is produced, usually after the third year, the characteristic grouping no longer prevails. These leaves, and others produced subsequently, appear to have their plastids distributed in the surface cytoplasm just as in the normal type. The plastids still remained grouped in the leaves produced during the first 3 years. The same plant may therefore have what may be termed juvenile leaves with grouped plastids and adult leaves with plastids normally distributed. Forty such juvenile leaves may be present with grouped plastids. In the epidermal cells of these adult leaves occur leucoplasts which still remain grouped, but this is the only evidence of such condition.

No spores have been obtained from the juvenile leaves but only from the adult. Thus far spores have been obtained from cultures 121 and 188 of the grouped plastid type. Prothallia produced from these spores are entirely normal in appearance. The grouped characteristic which has persisted—and still persists in prothallia derived from the original prothallium with grouped plastids—disappears in the sporophyte when it is 2–3 years old and does not reappear in the new gametophyte.

Little can be said at present regarding the cause of this loss. The geneticist would perhaps be inclined to consider that a remutation had occurred, or that the expression of the gene or genes controlling plastid behavior is modified by the internal environment of the adult leaves.

The grouping of plastids appears to be similar to an agglutination phenomenon. It is conceivable that a substance is produced in the cell by X irradiation which is responsible for the grouping, and that as later stages of the sporophyte are reached this agglutinating substance is dissipated or no longer produced.

In the case of the giant plate type (no. 262) the sporophyte generation again exhibits the giant plate type of plastid. These remarkable plastids are therefore transmitted as a result of sexual reproduction to the sporophyte and to the succeeding gametophyte. This inheritance

would be in accordance with the usual genetic behavior. But even here the plastid type could be independent of the gene. In other cases there appears to be a partial reversion of abnormal types to normal, but with some individuals retaining the abnormalities. These also tend to confirm the view of independent behavior. These cases will be considered in a subsequent paper.

From a purely physiological point of view, plastid mutation should occur as a result of X irradiation. If it is assumed that there is continuity of plastids from one generation to another, such mutations should become permanent. How these mutations could occur is of course still in the realm of speculation. In the spore the plastids are present as plastid primordia in the cytoplasm. The exact number that may be present has not as yet been definitely determined. The number of plastids in the basal cell of the protonema is generally ten or less, and it is probable that this is the number of plastid primordia in the original spore. These plastid primordia are protoplasmic; and as indicated by the work of GRANNICK (**7**), chloroplasts are high in protein. It has been demonstrated by NORTHRUP (**20**) that crystalline pepsin is deactivated by $\beta$ rays from radium and that other enzymes are deactivated by exposure to rays of high frequency. Flocculation is an obvious effect of high frequency (**6**). The significant point is that proteins are changed, and since proteins constitute a large proportion of the plastid body, changes in the protein would probably bring about changes in the plastid. Of course changes in the proteins of the plastid may be only incidental to other chemical changes occurring there. Equally pertinent to the mechanism of plastid mutation is the interesting concept of FRICKE that the points of primary activation by X rays and the points of chemical reaction are usually separated in space. If in the spore the chemical activation is in the cytoplasm, it is possible that all proplastids would be modified, and this could account also for the grouping of plastids previously described.

It seems a reasonable supposition that the extraordinary changes in plastids may be associated with changes in the protein of the plastid. With these protein modifications, changes in surface tension and viscosity would follow, with change in form and size resulting. A new condition once established continues by plastid divi-

sion. This presupposes also that the altered substances, be they proteins or other substances, are resynthesized in the pattern of the altered material. This is probably analogous to the multiplication of a virus once it is established in a suitable host.

Further studies of a physiological nature are to be undertaken with these plastid types. Their photosynthetic efficiency should provide information of interest. Studies on the inheritance of these plastid types is progressing and it is expected that results of these latter studies may be published soon.

## Summary

1. The spores of *Polypodium aureum* may be treated with calcium hypochlorite up to 50 minutes at least, with no deleterious effect on germination and growth.

2. Irradiation of spores with dosages as high as 30,000 r units is without effect on the percentage of germination. With higher dosages the percentage of spores germinating decreases. The growth rate is decreased with dosages as low as 10,000 r units, and progressively decreases with increased dosage.

3. From seventy-two cultures in which were sown nearly 300,000 spores, fifty-one prothallia having abnormal chloroplasts were isolated. Twenty-seven prothallia survived. These have been classified according to the characteristics of the chloroplasts. Including the normal, eleven types were noted. These prothallia have been propagated vegetatively during a period of 7 years. The plastid types persist.

4. Certain types are transmitted to the sporophyte as a result of sexual reproduction and retransmitted to the new generation of the gametophyte.

5. The grouping of the plastids (type B) disappears in the adult leaves of the sporophyte and does not reappear in the new gametophyte generation.

6. On theoretical grounds it is suggested that the plastid changes may be independent of any genic changes, and that certain plant mutations may be due solely to plastid changes.

CORNELL UNIVERSITY
ITHACA, NEW YORK

## LITERATURE CITED


1. BIEBLE, R., Die Wirkung der d. α Bestrahlung auf Protoplasma und Chloroplasten. Protoplasma **24**:215. 1935.
2. BOWEN, R. H., Studies on the structure of plant protoplasm. II. The plastidome and pseudochondriome. Zeitschr. Zellforsc. Mikrosk. Anat. **9**:1–65. 1929.
3. DOBZHANSKY, T., Induced chromosomal aberrations in animals. *In* DUGGAR, B. M., Biological effects of radiation. McGraw-Hill, New York. Vol. II. pp. 1167–1208. 1936.
4. GOODSPEED, T. H., and UBER, F. M., Radiation and plant cytogenetics. Bot. Review **5**:1–48. 1939.
5. FRICKE, HUGO, The chemical properties of X ray activated molecules with special reference to the water molecule. Cold Spring Harbor Symposia on Quantitative Biology **3**:55–65. 1935.
6. ———, The denaturation of proteins by high frequency radiations. Cold Spring Harbor Symposia on Quantitative Biology **6**:164–170. 1938.
7. GRANICK, SAM, Chloroplast nitrogen of some higher plants. Amer. Jour. Bot. **25**:561–567. 1938.
8. HRUBÝ, K., Durch X Strahlen hervorgerufene abnorme Plastiden. Soc. Roy. Lettres Sci. Bohême. Mem. 1935, Sec. XV. 1935.
9. IMAI, YOSHITAKA, The behavior of the plastid as a hereditary unit. The theory of the plastogene. Cytologia, Fujii Jubilee Vol. pp. 934–947. 1937.
10. JOHNSON, EDNA L., Effect of X rays upon green plants. *In* DUGGAR, B. M., Biological effect of radiation. McGraw Hill, New York. Vol. II. pp. 961–1013. 1936.
11. KNUDSON, LEWIS, Physiological study of the symbiotic germination of orchid seed. BOT. GAZ. **79**:345–379. 1925.
12. ———, Giant plastids in ferns produced from X-rayed spores. Amer. Jour. Bot. **21**:712–713. 1934.
13. ———, Has the chloroplast a semipermeable membrane? Amer. Jour. Bot. **23**:694. 1936.
14. KÜSTER, E., Beiträge zur Physiologie und Pathologie der Chloroplasten. Protoplasma **2**:65–69. 1917.
15. ———, Pathologie der Pflanzenzelle. Teil II: Pathologie der Blastiden. Berlin. 1937.
16. LANDER, C. A., The relation of the plastid to nuclear division in *Anthoceros laevis*. Amer. Jour. Bot. **22**:42–51. 1935.
17. LIEBALDT, ERNA, Über die Wirkung wässeriger lösungen oberflächenaktiver Substanzen auf die Chlorophyllkörner. Zeitschr. Bot. **5**:65–113. 1913.
18. LINDSTROM, E. W., Hereditary radium induced variation in the tomato. Jour. Heredity **24**:129–137. 1933.
19. NADSON, G. A., and ROCHLIN, E. J., L'effet des rayon X sur la protoplasmae, le noyau et le chondriome de la cellule végétale d'apres les observations sur le vivant. Protoplasma **20**:31–41. 1933.

20. NORTHRUP, J. H., Crystalline pepsin. VI. Inactivation by beta and gamma rays from radium and ultra violet light. Jour. Gen. Physiol. 17:359–363. 1934.
21. PACKARD, CHARLES, Biological dosimeters in radiology. Cold Spring Harbor Symposia on Quantitative Biology 2:264–273. 1907.
22. PRIESTLEY, J. H., and IRVING, A. A., The structure of the chloroplast considered in relation to its function. Ann. Bot. 21:407–413. 1907.
23. RANDOLPH, F., Cytology of chlorophyll types of maize. BOT. GAZ. 73:337–375. 1922.
24. SHULL, C. A., and MITCHELL, J. W., Stimulative effects of X rays on plant growth. Plant Physiol. 8:287–296. 1933.
25. SHARP, L. W., Introduction to cytology. 3d ed. New York. 1934.
26. SINNOTT, E. W., and DUNN, L. C., Principles of genetics. 3d ed. New York. 1939.
27. SIRKS, M. J., Plasmatic inheritance. Bot. Review 4:113–131. 1938.
28. STADLER, L. J., Induced mutations in plants. *In* DUGGAR, B. M., Biological effects of radiation. McGraw Hill, New York. Vol. II. pp. 1263–1280. 1936.
29. STONE, WINONA E., The origin, development and increase of chloroplasts in potato. Jour. Agr. Res. 45:421–435. 1932.
30. WEIER, ELIOTT, The structure of the chloroplast. Bot. Review 4:497–530. 1938.
31. WILSON, J. K., Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. 2:420–427. 1915.

# SPORE GERMINATION AND VEGETATIVE STAGES OF THE GAMETOPHYTES OF HYMENOPHYLLUM AND TRICHOMANES

ALMA G. STOKEY

(WITH THIRTY-FIVE FIGURES)

## Introduction

In 1843 TASCHNER (**23**) gave a short account of the germination stages and young gametophytes of *Trichomanes schmidianum* and *T. capillatum*, based on material collected in the indusia of dried specimens. PRESL (**20**) in the same year published a few drawings of germinating spores but no later stages. Later METTENIUS (**18**), in connection with an account of the sporophyte, gave an extensive account of the gametophytes of *Hymenophyllum* and *Trichomanes*, based largely on dried herbarium material. He examined the germination stages of more species than has anyone since his time. He also studied *H. tunbridgense* in culture. PRANTL (**19**) described and gave figures of early stages of *H. elegans*, *T. speciosum* (*T. radicans* Sw.), and *T. schmidianum*. JANCZEWSKI and ROSTAFINSKI (**17**) discussed the mature prothallia, sex organs, and embryo of *H. tunbridgense*. GOEBEL (**12**) described early stages of the gametophyte obtained in cultures of *H. dilatatum*, *T. diffusum*, and *T. palmatifidum*, and of field collections in Java of older stages of *H. dilatatum*, *H. eximium*, and *H. smithii*. BOWER (**1**) described the mature gametophytes of *T. pyxidiferum* and *T. alatum* from material growing in the greenhouses at Kew and Edinburgh, and gave an account of aposporous and apogamous growth in *T. alatum*. Later he gave a brief account of apospory in *T. kaulfussii* Hk. and Grev. (**2**). GIESENHAGEN (**11**), in a discussion of the Hymenophyllaceae, described some gametophytes chiefly from collections made by GOEBEL. SADEBECK (**21**) discussed and illustrated the germination of *H. rarum* and an undetermined species of *Trichomanes*. GEORGEVITCH (**9, 10**) reported on *T. kaulfussii*, with special reference to apogamy, apospory, and gemmae formation. HOLLOWAY (**16**) described the experi-

mental culture of the gametophytes of *H. pulcherrimum* and *T. reniforme*, and included results of field observations on several species of *Hymenophyllum* and *Trichomanes*.

## Material and methods

This study is based on cultures of seven species of *Hymenophyllum* and four of *Trichomanes* collected at Tjibodas and Lebak Saät, Java, early in July, 1937, and on a culture of *H. blumeanum* started in Formosa in 1931. The spores were collected from fresh leaves on smooth white paper and were planted within an hour or two after being shed. The early stages were obtained from cultures on sterilized decayed wood, from which it is easy to remove the young gametophytes. Several of the cultures on wood flourished for several months but eventually succumbed to infection by fungi. In a few cases early stages were also obtained from spores which had fallen into the indusium and germinated there. The later stages were obtained from cultures on peat by methods described in a previous paper (**22**). It may reasonably be inferred that conditions were favorable, since cultures of several species have proceeded to the stage of producing sex organs.

For a period of over six weeks the cultures were kept on a table near the window in the Treub Laboratorium, Buitenzorg, where they received approximately one-half the diffused light available. A few rough tests were made to ascertain a favorable intensity of light by comparing the growth of four sets of cultures of *H. holochilum*, *H. javanicum*, and *T. auriculatum* at different distances from the window. The intensity of light was measured by a metrophot. Station A was close to the window, where there was the maximum diffused light available in the laboratory; B, a table with one-half the light at A; C, with one-tenth the light at A; and D, with one-twentieth the light at A. In a 24-day test there was no difference in type of growth and very little in rate. This is, however, a short period for prothalli which grow as slowly as those of the Hymenophyllaceae. The slight advantage, more marked in *T. auriculatum* than in the more slowly growing species of *Hymenophyllum*, was in favor of those at station B where they received one-half the diffused light available through the window, with those at C (receiving one-tenth the maximum light)

next in rate of growth. These results, although meager and lacking in precision, indicate that the gametophytes of *Hymenophyllum* and *Trichomanes* are affected less by differences in intensity of light than are those of most leptosporangiate ferns. Their behavior in crowded cultures also suggested this.

A few rough tests were made on the length of viability of the spores of *H. javanicum* and *T. maximum*. The spores were collected on glazed paper and the paper folders were kept in a tin box at room temperature at the Treub Laboratorium. After thirteen days about half the spores of *H. javanicum* were still alive; a few germinated when nineteen days old, but none after that. Spores of *T. maximum* were not viable after six days. Undoubtedly the spores would retain their viability for a longer period with a more careful technique in handling them.

After six weeks' growth in Java the cultures were brought to the United States. Further growth was followed in South Hadley, Massachusetts, where the cultures grew in the north window of a laboratory during the college year. During the three summer months they were at the Marine Biological Laboratory, Woods Hole, Massachusetts.

## Investigation

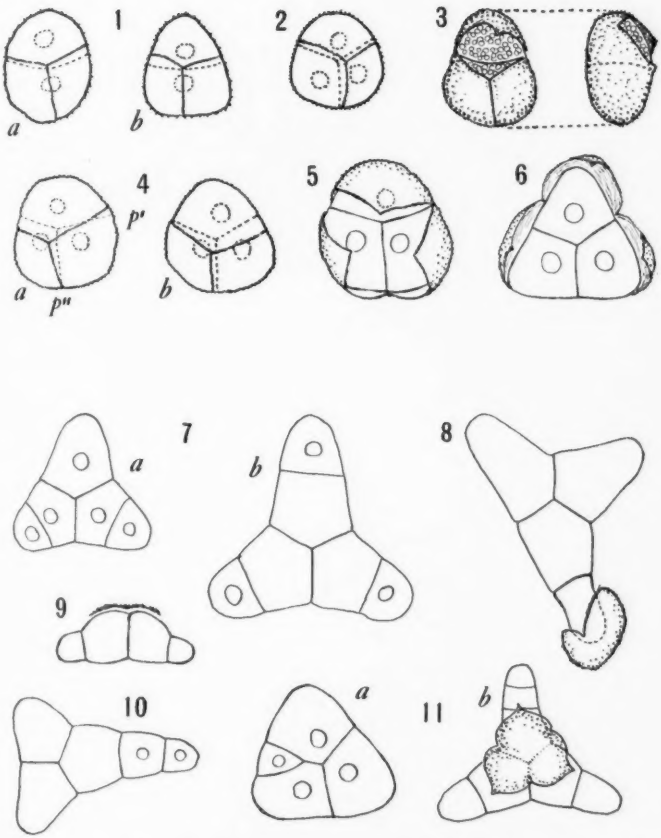
### Hymenophyllum

Cultures of seven species of *Hymenophyllum* were started in Java. After two years the following are still flourishing: *H. acanthoides* Ros., *H. holochilum* (v.d.B.) C. Chr., *H. kurzii* Prantl, and a form which Dr. Posthumus said was probably *H. blumeanum* Spr. and which will be referred to by that name. *H. junghuhnii* v.d.B. was in culture only a few weeks; *H. fuscum* (Bl.) v.d.B. for eight months, and *H. javanicum* Spr. for eighteen months. A culture of *H. blumeanum* started in Formosa in 1931 is still flourishing after eight years, during which time transfer cultures have been made about once a year.

The spores of all these species are of uniform type, spherical or tetrahedral, with contents strikingly green owing to abundant chloroplasts, and with a thin papillate coat on which the ridges are conspicuous. In all seven species the spores germinated within the

sporangium with the division into two or three cells (figs. 1, 2). This occurs in all the species of *Hymenophyllum* investigated. In most cases when discharged from the sporangium the spore showed the characteristic triradiate division into three cells, but cases of two cells were not uncommon, and in some cases there were three nuclei but two were not separated by a wall. The first wall divides the spore into two approximately equal cells, one of which broadens more than the other (fig. 1) and is then divided by a wall which meets the first in the center of the spore (figs. 2, 4). The growth is such that the gametophyte takes on the form of a triangular cushion consisting of three cells separated by walls making approximately equal angles (fig. 4*b*). The position of the walls is such that they have been referred to as three walls which meet in the center, although METTENIUS gave a figure of the two-celled stage and pointed out that the walls do not arise simultaneously. It is often possible to distinguish the order of formation of the two walls (which will be referred to as primary walls), since the angle made by the bending of the first wall may remain for some time larger than the other two angles (fig. 4*a*) and the constriction on the outer wall may be more marked than that produced by the second wall. The triradiate walls lie under the ridges of the spore coat (figs. 2, 4); and after its rupture, which may occur within a few hours after planting, the three arms of the young prothallus grow in the direction of the longitudinal axes of the valves (figs. 5, 6, 11*b*). The three cells then enlarge noticeably, and much larger and sharper tips are formed (figs. 6, 8). Secondary divisions of the cells may not occur for several days, five to ten days in the cultures at Buitenzorg. This is slow in comparison with the rate of division in gametophytes arising from chlorophyll-bearing spores in other ferns, for example, *Osmunda* spp., *Todea barbara*, and *Onoclea sensibilis*, or even in comparison with the development of most leptosporangiate prothalli after germination.

The secondary walls, which cut off the three points or arms, follow in sequence (fig. 7), and the three may be complete within seven or eight days, or not until twelve to fifteen, or even longer (fig. 7). Prothalli with an abundant supply of chloroplasts and every visible indication of vigor may remain for a long period with surprisingly little activity in the production of new cells. In a rather crowded

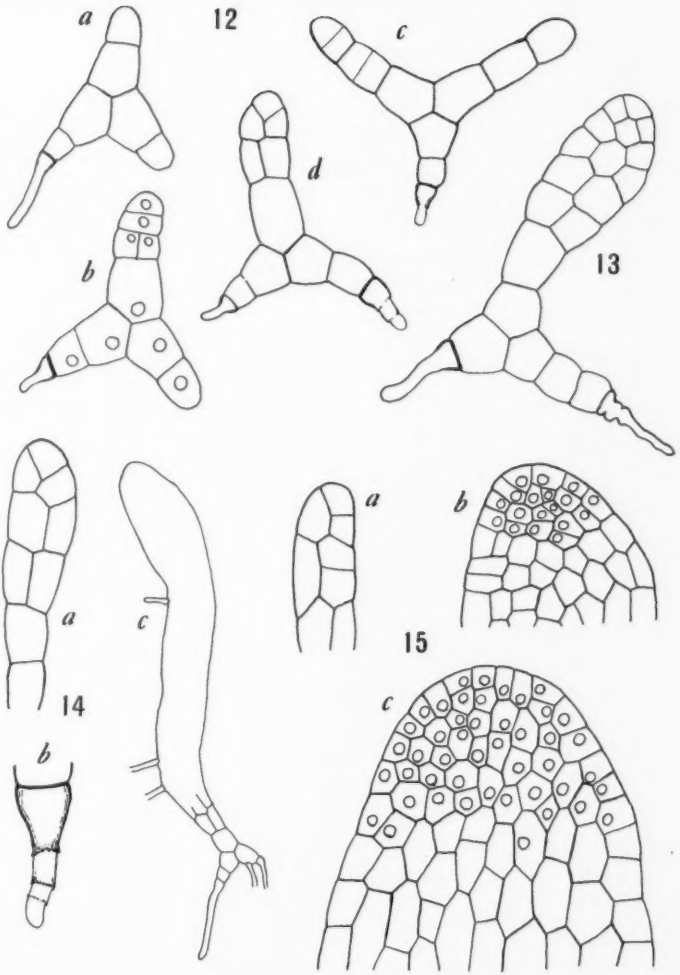



FIGS. 1–11.—Germination stages of *Hymenophyllum*. Fig. 1, *H. kurzii*, freshly discharged spores with first primary wall. Fig. 2, *H. acanthoides*, same with both primary walls. Fig. 3, *H. javanicum*, two views of germinating spore with first primary wall; 2 days old. Fig. 4, *H. acanthoides*, spores 3 hours after discharge from sporangium: *p'*, first primary wall; *p''*, secondary primary wall. Fig. 5, *H. javanicum* 1 day after planting. Fig. 6, *H. acanthoides* 7 days after planting. Fig. 7, same 8 days after planting. Fig. 8, *H. junghuhnii* 21 days after planting; position of spore coat unusual. Fig. 9, *H. holochilum*, side view of 6-celled prothallus. Fig. 10, *H. acanthoides* 21 days after planting. Fig. 11, *H. blumeanum: a*, unusual division in freshly shed spore; *b*, 21 days after planting; the three tips are in usual position with reference to valves of spore coat.

culture of *H. holochilum* nearly four months old, many prothalli were found which had only seven to ten cells and one or two rhizoids; but the cells had a full complement of chloroplasts and there was no suggestion of the depauperate aspect usually shown by retarded prothalli of other ferns grown in crowded cultures or in weak light. Healthy bright green prothalli of *H. acanthoides* consisting of only six cells were found in cultures five months old. The ability to remain vigorous in crowded cultures for so long is probably related to their adaptation to a wide range of light intensity. Irregularities, such as the production of a four-celled plate, were found occasionally (fig. 11*a*). Certain other irregularities occasioned by the failure of the second primary wall to develop will be discussed later.

Each of the three tips of the gametophyte grows independently into a rhizoid or a green filament. The branch most favorably placed continues as a filament for a short period and one or both of the others will be checked by the formation of a rhizoid which is usually terminal. When division in one arm is checked, the others may continue as in figure 12*c*, or even until there are longitudinal divisions in both branches; but ultimately one dominates and the other is checked. Although the early stages have a remarkably high degree of uniformity, subsequent development is varied, and, as noted by HOLLOWAY (**16**), "there is here no constant sequence in development such as is found in the fern gametophyte of the cordate type." Growth of the primary filament or filaments is usually apical but may be intercalary also in the early stages (fig. 12*c*). Longitudinal divisions appear first in the terminal cell, or less frequently in one farther back (fig. 12*b*). As all who have had *Hymenophyllum* gametophytes in culture have remarked, growth is very slow, not only in comparison with the quick germination but with the development of gametophytes of other leptosporangiate ferns. In the most rapidly growing culture, that of *H. acanthoides*, the largest prothallus noted had only twelve cells at the end of twenty-one days, and very few prothalli had more than twenty cells at the end of six weeks. In that time under similar conditions a polypod gametophyte might mature and produce a sporophyte.

Rhizoid development was late in all species. METTENIUS found rhizoid development belated in his cultures and in prothalli growing




FIGS. 12–15.—Fig. 12, *H. acanthoides:* *a*, *b*, 21 days old; *c*, 40 days old; *d*, 66 days old. Fig. 13, *H. holochilum*, 3 months old. Fig. 14, *H. acanthoides:* *a*, tip with apical cell, prothallus 3 months old; *b*, rhizoid from prothallus 26 days old; *c*, prothallus 5 months old. Fig. 15, *H. holochilum:* *a*, tip of branch 5 months old; *b*, tip with apical cell; *c*, tip with marginal meristem.

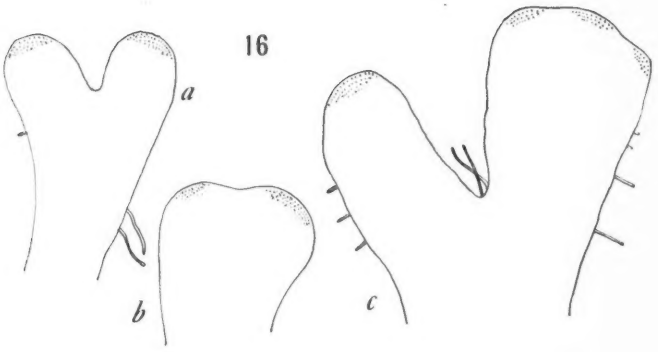
within the indusium, and gave figures of prothalli with seven to ten cells and no rhizoid. The earliest suggestion of a rhizoid was noted in *H. acanthoides* eight days after planting, but in most cases it was fifteen to twenty days. The first cell cut off from a tip may develop into a rhizoid (figs. 8, 13), but that is less common than the formation of a rhizoid from the second or even the third cell (figs. 12*a–d*, 13). The beginning of a rhizoid is indicated by the slender form of an elongating terminal cell or of a lateral papilla and by a change in the character of its contents. The young rhizoid on young plants is well provided with chloroplasts, which gradually grow smaller and paler, finally disappearing. The wall which separates the rhizoid from the green prothallial cell turns brown early, and the lateral walls begin to turn brown while there are still chloroplasts in the rhizoid. In a rhizoid which grows slowly there is often a colorless tip on a pale extension of the inner layer of the rhizoid wall (fig. 12*c*, *d*). In some cases there is a series of brown rims indicating where the inner layer has stretched beyond the inclosing layers (figs. 12*d*, 14*b*). Branching rhizoids are common, especially near the base of old prothalli. The branching rhizoid seems to be a general characteristic of the Hymenophyllaceae and is mentioned or illustrated in almost all accounts of the gametophyte, from TASCHNER'S to the present. Two rhizoids may arise from one prothallial cell; such cases are frequent, particularly near the base of old prothalli (figs. 14*c*, 17*b*).

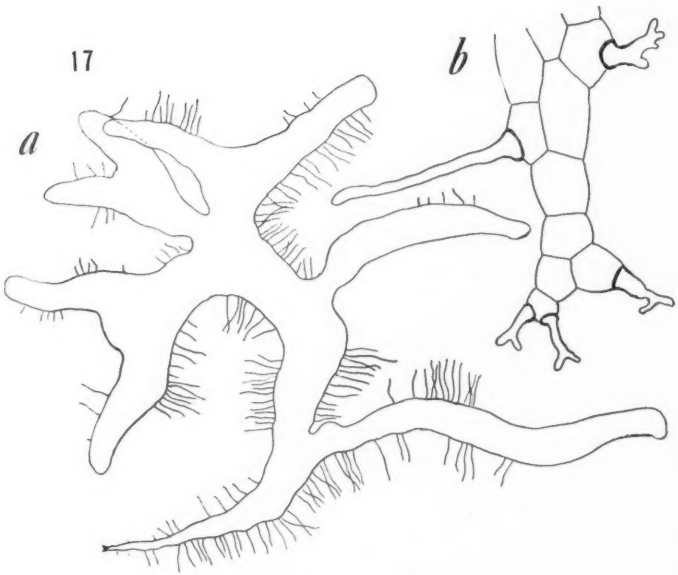
The broadening of one arm is usually brought about by the development of the terminal cell as an apical cell with two cutting faces (figs. 12*d*, 14*a*), but the behavior at the tip is not entirely like that of the apical cell of cordate prothalli, which gives rise to segments undergoing many divisions and considerable enlargement. At an early stage in the development of the ribbon-like prothallus the terminal cell may divide by a wall more or less oblique to the longitudinal axis; but the segments may not divide at all, or at most a few times, and in any case they enlarge very little (figs. 12*d*, 14*a*, 15*b*). The small number of cells formed by the segments and their limited growth prevent the attainment of a lobed tip. Sometimes the wall in the terminal cell is more nearly parallel to the axis and there is formed a tip in which is a series of vertical and cross walls (fig. 15*a*). In any case the apical cell, such as it is, is succeeded by a

group of meristematic marginal cells (fig. 15*c*). The tip remains rounded or blunt, even when the ribbon-like branch becomes twenty to thirty cells wide (fig. 16).

Branching is ordinarily brought about by cessation of the activity of some of the cells of the rounded meristematic apex with continued activity of the cells on both sides of the subsiding meristem (fig. 16*b*). Occasionally the checking of the activity of the central cells is followed by development of rhizoids in the stretch of margin between the new branches. If the cells which cease division are median, the two meristematic regions approximately equal, and the new branches grow at the same rate, the result is a dichotomy (fig. 16*a*). Or two regions at the tip may cease activity, leaving three meristematic areas which will result in three branches. Or, as is usually the case, the meristematic regions may be unequal; then the two branches may both elongate at much the same rate, making a strong and a weak branch, or one branch may lag and be left behind by the greater growth of the other, becoming a lateral branch (fig. 16*c*). As the branches lengthen they twist in such a way that they do not all lie in one plane, but form a rosette-like tuft. A characteristic gametophyte one year old of *H. holochilum* is shown in figure 17*a*. The original three primary cells were still present (fig. 17*b*), and the activity of horizontal branches which give rise to a succession of vertical branches had not yet begun. In these cultures the vertical branches were usually 9–12 mm. long and fifteen to thirty cells wide. The primary cells die after the prostrate branches become active, and none were found on prothalli two years old. Adventitious shoots may arise at any place on the margin of the mature portions of the thallus, but are found most frequently on the lower parts, particularly on the prostrate branches. They are produced freely and arise in the same manner as such branches on other ferns.

Rhizoids are abundant on the erect branches, even 10–12 mm. from the base (fig. 17*a*). They arise from the marginal cells only and usually grow toward the under side. All the rhizoids except those on the basal and lower parts of the branches stand far above the substratum and are far too short to reach it, as HOLLOWAY noted for *H. pulcherrimum* in culture and for that species and others in the field. It is not apparent that they are of any use to the gametophyte—un-




FIGS. 16, 17.—Fig. 16, *H. acanthoides*, tips of branches with position of marginal meristem indicated by stippling. Fig. 17, *H. holochilum: a*, prothallus 1 year old: *b*, base of *a*.

less they are effective in keeping space between the overlapping lobes and branches in crowded tufts. The presence of an endophytic fungus has been noted by various writers, but none was found in the cultures which were planted on sterile peat. The vigorous growth of the gametophytes with uninfected rhizoids gives support to the statement of HOLLOWAY (**16**) that "the fungal infection is only of minor importance in these gametophytes."

The branching ribbon-like gametophyte is the only type ordinarily associated with *Hymenophyllum*, although METTENIUS described that of *H. microcarpum* as consisting of filaments with profuse branching which suggested dichotomy. He described the gametophyte of *H. pulchellum* also as being prevailingly filamentous, with leaflike expansions two to five cells wide, an appearance similar to that of *T. sinuosum*. GOEBEL (**12**) raised the question of the identity of these gametophytes, since they differed so much from those which he himself had collected and from the others described by METTENIUS. As a third type, METTENIUS described the branching ribbon-like gametophyte of *H. elegans*. This is apparently the prevailing form in *Hymenophyllum*.

The gametophytes of *H. javanicum* (fig. 18) and those of *H. fuscum* suggest the aberrant filamentous type which METTENIUS described. The germination of these two species was like that of all other recorded species of *Hymenophyllum*, but growth was slower and development was of a different type from that described for *H. acanthoides*, *H. holochilum*, *H. kurzii*, and *H. blumeanum*. The filamentous development continued for many months, with abundant branching, so that each gametophyte made a compact rosette. The cells in both species were short and swollen, and there were many short stubby branches, but they did not suggest a dichotomous type. There was a strong tendency for the primary cells to make intercalary divisions and to produce extra rhizoids and filaments, although in other species of *Hymenophyllum* such activities are rare.

*H. fuscum* was in culture eight months and in that time no expansions were formed, although there were longitudinal divisions in a few cells. In cultures of *H. javanicum* sixteen months old most of the branches were of the filamentous type, but branches two cells wide

were found on a few prothalli although there was no indication of the formation of an active apical region (fig. 18*c*).

It is difficult to keep such forms in culture as they are more likely to be overcome by chance infections of algae or fungi than are those which grow more rapidly. It is possible that this unusual type of development should be attributed to unfavorable conditions, but it

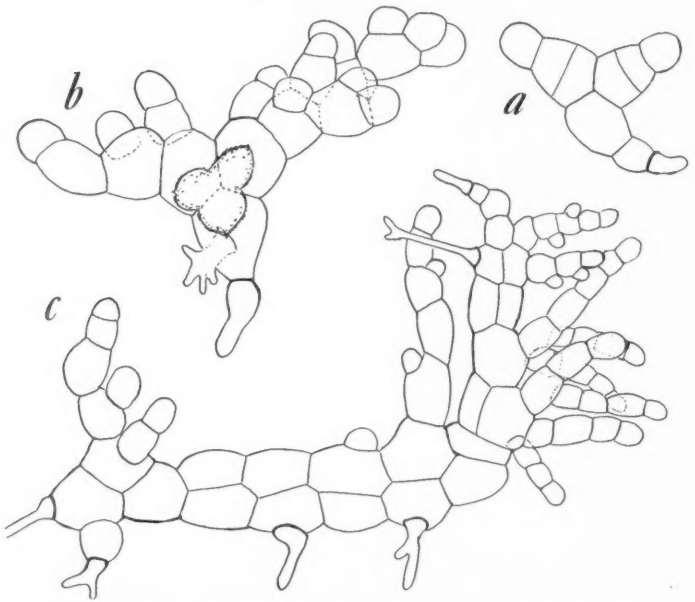



FIG. 18.—*H. javanicum*, stages in development of prothallus: *a*, 22 days old; *b*, 8 months old; *c*, 16 months old.

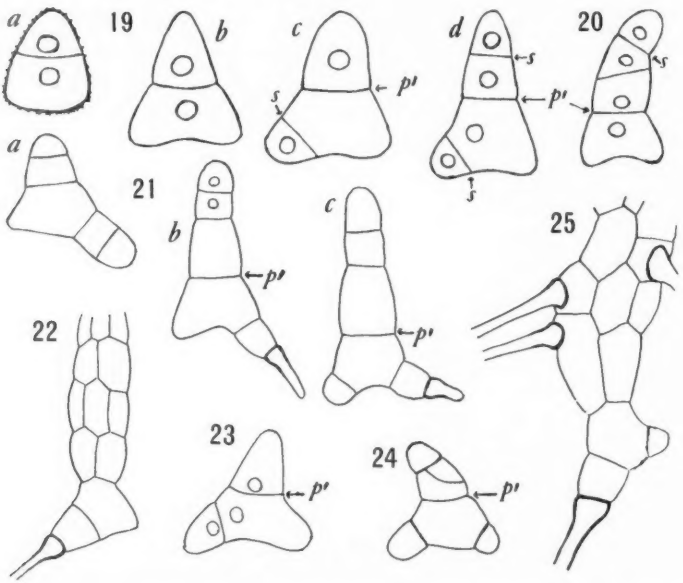
does not seem probable that such is the explanation. Individuals of other species which grew slowly did not follow this line of development. HOLLOWAY (**16**) found that the gametophyte of *H. pulcherrimum*, which ordinarily produced branching ribbons, tended to be filamentous when grown in specially moist conditions. The gametophytes of *H. fuscum* and *H. javanicum* were grown under the same conditions as those of the other species, and there was considerable variation in the amount of moisture in the peat at different times, although at no time were they very moist.

The gametophytes of *H. holochilum* and *H. kurzii* began to bear sex organs when about twenty months old. A few scattered antheridia were found near the margin, with archegonia singly or in twos on the same gametophyte. COPELAND (**8**) includes *H. kurzii* in *H. holochilum*. Although the cultures of *H. acanthoides* grew more rapidly than any of the other species at the beginning, neither that nor the Java cultures of *H. blumeanum*, both two years old, have as yet produced sex organs. A culture of *H. blumeanum* which was started in Formosa in August, 1931, is still flourishing. When nearly four years old it began to bear archegonia and has continued a sparing production of them at intervals ever since. No antheridia have been found in the cultures.

An interesting variation in germination was found in *H. acanthoides*, *H. holochilum*, *H. kurzii*, *H. fuscum*, and *H. javanicum*. It occasionally happens that the second of the primary walls fails to develop and the typical radiate three-celled stage is not attained. Figure 19*a* shows a form which is occasionally found on the rupture of the sporangium, and at this stage it cannot be determined whether the second wall is belated or suppressed. Figure 19*b*, however, shows a prothallus of *H. holochilum* sixteen days old in which the second wall has probably been suppressed, as ordinarily the division into three cells takes place shortly after the spores are discharged, if (as is usual) it has not already taken place in the sporangium. When the secondary primary wall does not appear, the first will remain flat and does not assume its characteristic angle; but for a short period at least it lies not far from the center of the original cell. When the second of the primary walls is suppressed, growth of the three points may proceed in the usual way, but in general there is less uniformity of development. The two tips belonging to the undivided cell enlarge less than the other, and frequently one of these remains inactive and does not cut off a peripheral cell.

The primary wall ($p'$) is considerably broader than the secondary wall ($s$), often twice as broad. The primary cell cut off by the primary wall may undergo secondary divisions before the peripheral divisions take place in either of the other two arms (fig. 20), as sometimes happens in prothalli with typical germination (fig. 10). In the gametophytes represented in figures 21*a*, *b*; 22, and 23 the secondary walls in the third tip are overdue, according to the usual rate of de-

velopment, and they are probably cases in which not only is one primary wall suppressed, but also the secondary wall in one tip. In the usual type of germination it is rare to find a gametophyte which




FIGS. 19–25.—Prothalli with first primary wall present but second suppressed: *p'*, first primary wall; *s*, secondary wall. Fig. 19, *H. holochilum: a*, freshly discharged spore with first primary wall; *b*, prothallus 16 days old; *c*, 16 days old, secondary wall in one tip; *d*, 16 days old with two secondary walls. Fig. 20, *H. acanthoides*, 21 days old, primary and secondary walls in one tip; intercalary division; two tips without secondary walls. Fig. 21, same: *a*, 3 months old, secondary walls in two tips; *b*, 26 days old, secondary walls in two tips (one undivided); *c*, 57 days old, secondary walls in all tips. Fig. 22, *H. kurzii*, base of prothallus 5 months old, one tip without secondary wall. Fig. 23, *H. fuscum*, 12 days old. Fig. 24, *H. javanicum*, 51 days old, secondary walls in all tips; intercalary wall in primary cell. Fig. 25, *H. holochilum*, base of prothallus 1 year old; late stage of prothallus like fig. 21*c*.

has reached the plate stage without at least one division in the other tips of the original three-celled plate, but it is much less rare in the type under discussion. In figure 22 is shown a gametophyte which is evidently a later stage of one which began like the one in figure

21*b*; figure 25 shows one which probably began like that in figure 21*c*. The relation of this type of germination to that of *Trichomanes* will be discussed later.

## Trichomanes

Four species of *Trichomanes* were studied. *T. auriculatum* and *T. bilabiatum* have been in culture for two years and have reached the stage of bearing sex organs. *T. maximum* has also been in culture for two years but it has grown much more slowly and has borne

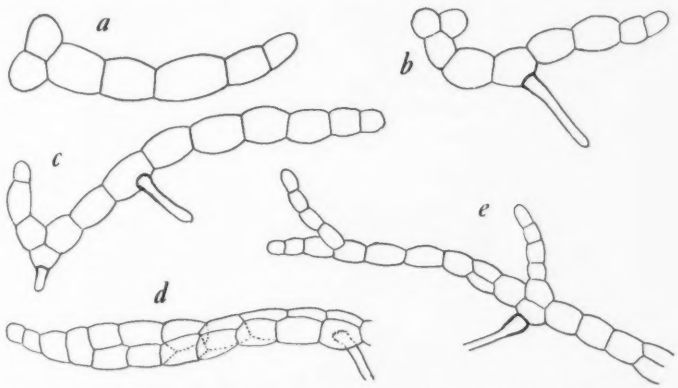



Fig. 26.—*Trichomanes nitidulum: a, b, c,* prothalli 4 months old; *d,* 8 months old; *e,* branch from prothallus 1 year old.

antheridia but not archegonia. *T. nitidulum* was in culture for twenty months.

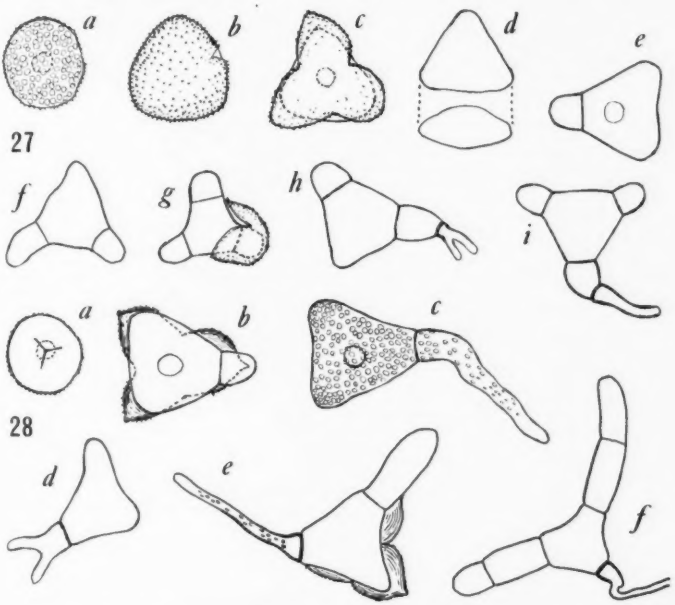
*T. nitidulum* in its germination follows the type of *Hymenophyllum* in the formation of three cells separated by radiating walls (fig. 26*a–c*). This type was recorded by Mettenius for *T. ampliatum*, *T. crispum*, and *T. reniforme*. Giesenhagen (**11**, p. 422) was in error in stating that Mettenius thought that only one type of germination was found in *Trichomanes*, since Mettenius (**18**, p. 491) expressly states that the three species mentioned agree with *Hymenophyllum* in mode of germination and are unlike *T. schmidianum*, *T. olivaceum*, and *T. humile*, in which a small cell is cut off from each tip by a tangential wall. Goebel (**12**) recorded the 3-celled type for *T. palmatifidum*, and Campbell (**5**) for *T. draytonianum*. The three

primary cells in *T. nitidulum* may give rise either to filaments or to rhizoids, as in *Hymenophyllum*, but in these cultures there was frequently considerable delay in the development of one or two of the tips (fig. 26*a*, *b*). In its subsequent growth the prothallus does not follow the usual *Hymenophyllum* development, but forms branching filaments. In cultures eight months old longitudinal divisions were found in single cells and in sections of filaments (fig. 26*d*), but no true ribbon-like expansions with apical broadening at the tip were found in cultures twelve to twenty months old. The prothalli showed considerable similarity in habit to those of *H. fuscum* and *H. javanicum*, although there was a difference in aspect because of the greater elongation of the cells and filaments in *T. nitidulum*.

The spores of *T. auriculatum*, *T. bilabiatum*, and *T. maximum* germinate in the manner usually associated with *Trichomanes*, or a modification of it. The spherical spores are deep green with abundant chloroplasts, and the tripartite ridges on the thin papillate spore coat are short and inconspicuous (fig. 28*a*). Germination in the sporangium was not found in these species. TASCHNER, however, found in *T. schmidianum*—which in other respects is similar to these three species—that the spores may germinate within the sporangium, and he gave a figure of the two-celled stage. Cracking of the spore coat usually occurs within a day or two after the planting of the freshly discharged spores.

In *T. auriculatum* and *T. bilabiatum* a triangular cushion-like body develops after the rupture of the spore coat and before there are any septations (fig. 27*b–d*). As in *Hymenophyllum*, each tip of the spore ordinarily is in line with a valve of the spore coat (fig. 27*c*), and the growth from each tip follows the longitudinal axis of the valve (fig. 28*b*, *e*). If, as occasionally happens, the crack is shallow or the spore is crowded as it germinates, the spore coat may be found as a cap on one tip of the thallus (fig. 27*g*). Within two to four days the first wall may appear and cut off one of the tips (figs. 27*e*, 28*b*); a second tip is then cut off (fig. 27*f*), and ultimately a third (figs. 27*g*, *i*; 28*f*), leaving an undivided central cell. This will be referred to as the *Trichomanes* type of germination, since so far as is known it is found in no other genus. In the case of spores germinating and continuing growth within the indusium, on which much of the early work was

based, the three peripheral cells are more likely to be of the same size for a period after the tangential walls appear than in the case of spores germinating under conditions more favorable for growth. Stages such as that in figure 27*g* were found within the indusium or in cultures with slow growth, twenty-five to thirty-five days after

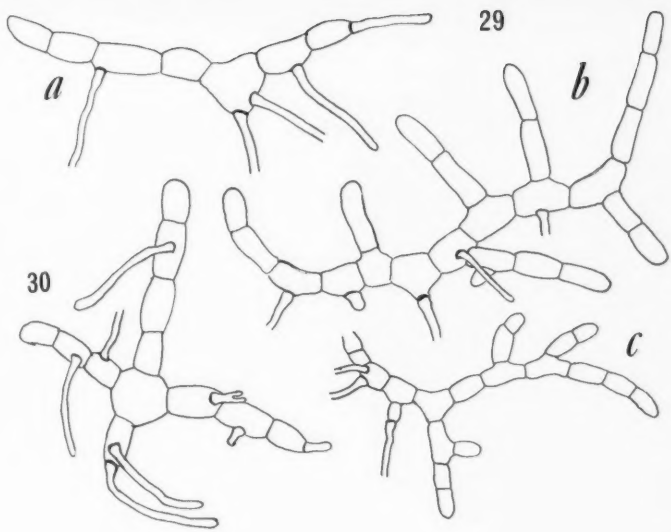


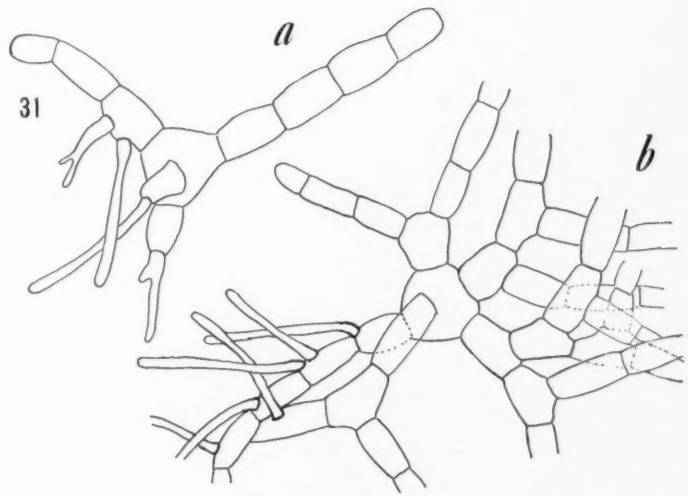

FIGS. 27, 28.—Fig. 27, *T. bilabiatum: a*, fresh spore; *b–d*, stages in germination before appearance of walls; *d*, 4 days old, two views; *e*, 4 days old; *f*, 5 days old; *g*, prothallus germinated within indusium, position of spore coat unusual; *h*, 34 days old; *i*, 43 days old. Fig. 28, *T. auriculatum: a*, fresh spore, ×300; *b*, 2 days old; *c*, 5 days old; *d*, 17 days old; *e*, 9 days old; *f*, 24 days old.

planting. In cultures on wood, where conditions were favorable, the second wall usually appeared when the prothallus was five to ten days old (fig. 27*f*), and the third at fifteen to twenty days, when the first arm and even the second had made noticeable growth. The growth of these cultures indicates that the more favorable the conditions for growth, the more variable and the less symmetrical the young gametophyte. Germination in *T. bilabiatum* was in general

more symmetrical than in *T. auriculatum*. This may be due solely to its slower growth, or it may indicate that it is nearer to the central type of the genus. In *T. bilabiatum* the first rhizoid usually arises from the second cell formed from a tip, but in *T. auriculatum* the first cell cut off usually grows into a rhizoid (fig. 28*c–e*). Rapid growth of the first rhizoid usually prevents the attainment of the symmetrical 4-celled stage, although by the formation of walls in the three tips an undivided central cell is formed (fig. 28*f*). In *T. bilabiatum* the wall cutting off the third tip is sometimes belated, but no old prothallus was found in which the wall was suppressed. In *T. auriculatum* it is even more likely to be belated, but the third tip usually develops with considerable vigor in the course of time. The central cell remains green and retains its power to divide for many months. At a later period it usually gives rise to one or more filaments or rhizoids in addition to the three growths arising from the tips of the original triangular cushion. Gametophytes three to five months old usually had one or more rhizoids or green filaments arising from the central cell, in addition to the three growths derived from the tips of the spore. This tendency to form extra filaments and rhizoids was more marked in *T. bilabiatum* than in *T. auriculatum*, and gametophytes of the former species four to five months old usually had four or five filaments arising from the central cell (figs. 30, 31).

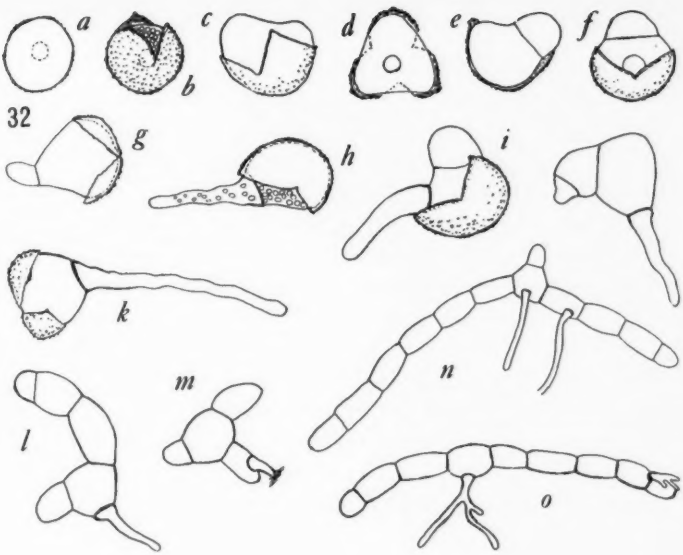
The mature gametophytes of both *T. auriculatum* and *T. bilabiatum* are of the branching filamentous type with no longitudinal divisions in the vegetative cells. The cells of the former species were usually longer and more slender than those of the latter, giving the plants a slightly different aspect under the microscope, although the general appearance of both cultures was the same. Both were equally green and equally vigorous, and to the unaided eye looked like a fine and healthy green turf, the *Rasen* of METTENIUS and GOEBEL. Antheridia appeared on the gametophytes of both species about nine months after planting, and a few weeks later archegonia had developed. Sex organs were produced more abundantly on *T. bilabiatum* than on *T. auriculatum*, and during the summer months numerous archegoniophores were formed on pale prostrate branches toward the periphery of the tufts. Antheridia were produced sparingly and no swimming sperms were seen; no sporophytes had developed at the end of two years.

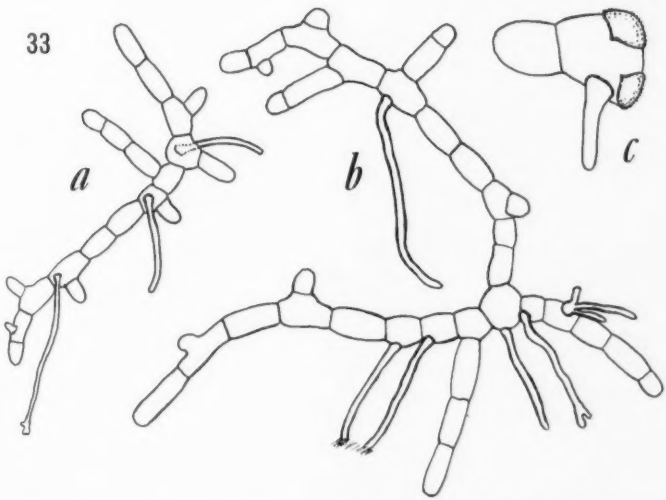



FIGS. 29–31.—Fig. 29, *T. auriculatum: a*, prothallus 51 days old; *b*, $3\frac{1}{2}$ months old; *c*, 4 months old. Fig. 30, *T. bilabiatum*, $4\frac{1}{2}$ months old. Fig. 31, *T. bilabiatum: a*, prothallus 3 months old; *b*, central portion of prothallus 5 months old; primary cell with five branches.

The spores of *T. maximum* are smaller than those of the preceding two species but are similar in aspect. Although cracking of the spore coat occurred within a day or two after planting, the first wall was not found in any gametophyte until ten days later (fig. 32*e–h*). Triradiate development was found (fig. 32*d*, *g*, *k*) but it was hardly as common as the less symmetrical development (fig. 32*e*, *f*, *h*). The first cell cut off develops as a rhizoid in many cases, and there is a definite tendency toward suppression of the third tip, so that the gametophyte frequently develops as a more or less lopsided filament (fig. 32*o*). The prothallus of *T. auriculatum* sometimes develops for a considerable period in this manner but the modification of the triradiate type is less pronounced. It has been suggested (**14**) that the type of germination found in *T. maximum* should perhaps be regarded as a third type in the family, because previous descriptions indicate that only one cell is cut off at germination and then a straight filament develops. While there is a straight filament in many cases, however, there are also examples of triradiate development (fig. 32 *l*, *m*, *n*). The cutting off of a single cell at germination, with considerable delay in the formation of walls in the other two tips, is probably much more common in *Trichomanes* than has been supposed. Germination in *T. maximum* and related species probably represents an extreme case. While the extreme forms of the early germination stages of *T. maximum* are unlike those of such species as *T. schmidianum*, *T. diffusum*, and *T. bilabiatum*, there are many gradations found in *T. maximum*—from forms with extreme modifications to such variations from the triradiate symmetry as are frequently found in *T. auriculatum* and brought about by delay in wall formation in one tip of the thallus. In *T. maximum* itself triradiate development is eventually attained in many cases (figs. 32 *l*, *m*, *n*; 33*a*, *b*). It is possible that in some cases, perhaps in many, the first rhizoid does not arise as a growth from one of the three tips of the spore, as in figure 32*j*, *k*, but as an extra growth from the central cell such as is frequently found in older prothalli of *T. auriculatum* (fig. 29*a*). This is apparently the case in a prothallus which at sixty-eight days consists of only two green cells and a rhizoid (fig. 33*c*). Triradiate development with an extra rhizoid arising from the central cell is of common occurrence in prothalli five to seven months old. Unfortu-




FIGS. 32, 33.—Fig. 32, *T. maximum: a*, fresh spore; *b*, 2 days after planting; *c*, *d*, *e*, 10 days old; *f*, 15 days old; *g*, 11 days old; *h*, 20 days old; *i*, 34 days old; *j*, *k*, 26 days old; *l–o*, 5½ months old. Fig. 33, *T. maximum: a*, *b*, prothalli 7 months old; in each the primary cell has given rise to three filaments and a rhizoid; *c*, 68 days old.

nately the series made was not close enough to determine how early an extra rhizoid may arise from the central cell. The growth of *T. maximum* was extremely slow, as was noted by GOEBEL (**13**), who kept it in culture three years without obtaining sex organs. The largest prothallus found in December, 1937, in a culture five months old, had only twelve green cells and two rhizoids (fig. 32*n*). The rate of growth then increased, and in February prothalli were found which had twenty-five to thirty-five green cells and two to five rhizoids (fig. 33*a*, *b*). The drawings given by PRANTL for the closely related species *T. speciosum* (*T. radicans* Sw.) are very similar to some of the early stages of *T. maximum*. The similarity extends also to the rate of growth, which he described as *ausserordentlich langsam*. In cultures five months old he found no prothallus which had more than eight cells and two rhizoids. The older gametophytes developed as branching filaments with no longitudinal divisions, and in general aspect were like the preceding two species. Antheridia were found on gametophytes one year old but no archegonia have appeared on cultures two years old.

The rhizoids of all four species of *Trichomanes* are similar to those of *Hymenophyllum* in their heavy brown walls and their frequent branching. On young prothalli chloroplasts are abundant in the young rhizoids and may persist until the rhizoid has attained considerable length (fig. 28*c*, *e*). The production of two rhizoids by one cell is of common occurrence (figs. 29, 30, 31), and it is especially characteristic of the cells of the pale prostrate branches associated with the production of archegoniophores.

The gemmae, which develop abundantly and form a notable feature of the gametophytes of *Hymenophyllum* and *Trichomanes* under certain undetermined conditions, were not found in any culture.

## Discussion

All who have worked with the Hymenophyllaceae, either with the sporophyte or with the gametophyte, agree that it is a natural family rather sharply set apart from other leptosporangiate ferns. Among recent workers, BOWER (**3**) recognizes two genera, *Hymenophyllum* and *Trichomanes*. COPELAND (**7**, **8**), dealing with the Old World genera only, recognizes three, returning *T. reniforme* to PRESL'S genus *Cardiomanes;* he states that further divisions of the

family are probably desirable but should be deferred for the present. CHRISTENSEN (6) also recognizes *Cardiomanes*, and adds the South American monotypic genus *Serpyllopsis*. It may be well to consider to what extent the gametophyte may contribute to an understanding of the family. Considering the size of the family our knowledge is very limited. There are few species for which we have both germination and mature stages. The present discussion will consider only the vegetative character of the gametophyte, which according to our present knowledge shows a greater departure than the reproductive from other fern types.

Germination stages are known for about thirty species of *Hymenophyllum* (12, 16, 18, 19, 21) and for scarcely half as many of *Trichomanes* (12, 16, 18, 19, 23). They are remarkably constant in *Hymenophyllum* but decidedly less so in *Trichomanes*. A striking peculiarity of the gametophyte is its tendency to assume the triangular form, which is seen in *Hymenophyllum* before the secondary walls appear, and may be very striking in *Trichomanes* before any walls appear. There is no ready explanation for the triradiate habit. The filamentous habit in the family may perhaps be correlated with the hygrophilous habit; but it is difficult to see why the hygrophilous habit in this family should result in such beautiful radiate symmetry of form when in other families the development of filaments is associated with irregularities of growth and a high degree of plasticity, such as is found in the higher families of ferns. It is indeed true that in the filamentous species of *Trichomanes* there is greater irregularity and a higher degree of plasticity; but probably the filamentous habit in that case has been derived from a type with the conservative and symmetrical structure of *Hymenophyllum*. The triradiate tendency is one of the things which, in the present state of our knowledge of formative influences, must be looked upon as "inherent."

Among other peculiar features of the gametophyte of the Hymenophyllaceae are the pittings in the lateral walls of the cells of the gametophyte, described by various investigators (11, 12, 17, 21); the highly specialized gemmae which apparently develop abundantly in nature (2, 10, 14, 18); the rhizoids with their strong tendency to branch, and the restriction in position to the marginal cells of the ribbon-like prothallus.

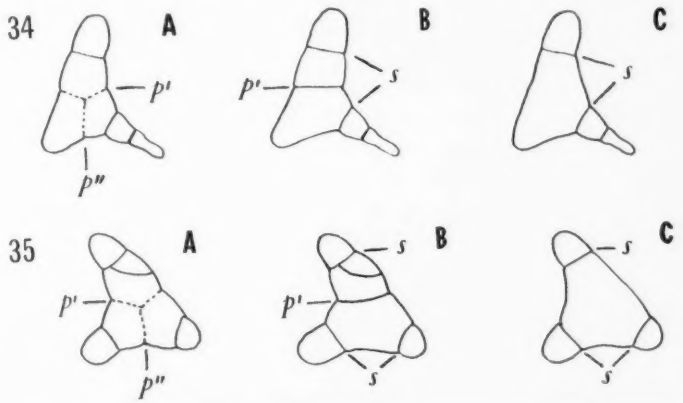
A general change in attitude toward the phylogenetic position of

the Hymenophyllaceae, and also of the relation of *Hymenophyllum* to *Trichomanes*, has occurred since PRANTL (**19**) characterized the family as *die niedrigste Entwickelungsreihe der Farne*. BOWER (**3**) has advanced reasons based on the morphology of the sporophyte for looking upon *Trichomanes* as being farther away than *Hymenophyllum* from the ancestral type. HOLLOWAY (**16**) arrived at the same conclusion from his study of the ecological habit and distribution of the New Zealand species. The greater plasticity and variability of the gametophytes of *Trichomanes* and the shorter time required for them to reach maturity suggest that *Trichomanes* is the younger and more specialized genus.

The relation of the two types of germination has long been a problem which has either been ignored or met by the statement that the walls which meet in the center of the germinating spore of *Hymenophyllum* no longer meet in *Trichomanes*, or vice versa (**14, 19, 21**). Such statements imply that the three walls which form in the tips of the triangular prothallus of *T. bilabiatum*—or a similar species—are homologous with the primary walls in *Hymenophyllum*. A serious objection to this interpretation is the fact that the primary walls in *Hymenophyllum* arise as two walls and not as three. It must be remembered that since the primary walls in the *Hymenophyllum* gametophyte arise under the tripartite ridges, the position of the primary walls is determined in the spore mother cell. The position of the three walls in the young thallus would not favor growth into the gaps made by the cracking of the spore coat, and therefore growth of the three tips proceeds along the longitudinal axes of the valves rather than into the gaps (figs. 5, 6). In *Trichomanes* there would be no structural difficulty in growth into the gaps, but even here the tips follow the longitudinal axes of the valves (figs. 27*c*; 28*b*, *e*; 32*d*). This suggests evolution from a form in which the direction of growth was determined by a condition which no longer exists. It is a strong reason for looking upon the 4-celled type of germination of *Trichomanes* as being derived from the 3-celled type of *Hymenophyllum*.

Certain types of germination which were found occasionally in several species of *Hymenophyllum* suggest a line of evolution from the 3-celled type of *Hymenophyllum* (and certain species of *Trichomanes*) to the 4-celled type of *Trichomanes*. This could be brought

about by the permanent suppression of the two primary walls which cause the triradiate division of the 3-celled spore. The two series of diagrams given in figures 34 and 35 suggest a probable sequence in evolution. The sequence in figure 34*A–C* is based on a camera lucida drawing (*B*) of a prothallus of *H. acanthoides* in which the first primary wall (*p'*) is present but not the second; secondary walls (*s*) have formed in the first primary cell and in one tip of the undivided




FIGS. 34, 35.—Diagrams suggesting evolutionary sequence of germination types in Hymenophyllaceae· *p'*, first primary wall; *p''*, second primary wall; *s*, secondary wall. Fig. 34*B*, *H. acanthoides*, 40 days old: *A*, diagram based on *B*, with usual arrangement of primary walls indicated by dotted lines; *C*, diagram based on *B*, with primary wall omitted. Fig. 35*B*, *H. javanicum*, 51 days old, ×150; *A*, diagram based on *B*, with usual arrangement of primary walls indicated by dotted lines; *C*, diagram based on *B*, with primary wall omitted; intercalary wall also omitted.

portion. The same prothallus is shown in *A*, with the primary walls as they would ordinarily develop indicated by dotted lines. Diagram *C* shows the type of prothallus which would be found if both primary walls were suppressed. It will be seen that *A* is similar to the drawing of the same species in figure 12*a*, and *C* to the prothallus of *T. bilabiatum* shown in figure 27*h*. A similar sequence is shown in figure 35, based on a camera lucida drawing (*B*) of a prothallus of *H. javanicum*, in which the first primary wall (*p'*) is present and there are secondary walls in all three tips. In *A*, as in figure 34*A*, the two pri-

mary walls ordinarily present are indicated by dotted lines; in *C* only the secondary walls are shown and the primary ones are omitted. (In *C* the intercalary wall present in the primary cell is not shown, as such walls are apparently limited to a few species of *Hymenophyllum* and are not found at all in *Trichomanes*.) Diagram *A* is not unlike figure 18*a* of the same species, while *C* suggests the prothallus of *T. bilabiatum* (fig. 27*g*).

There is no obvious ancestor for the type of gametophyte found in the Hymenophyllaceae. A strong similarity exists between the spores of this family and those of the Osmundaceae in form, chlorophyll content, and general aspect of the spore coat. There is also a degree of similarity in the early development of the plate. CAMPBELL (4) has figured a gametophyte of *Osmunda cinnamomea* with a triangular thallus not unlike an early 3-celled stage of *Hymenophyllum*, except for the rhizoid, and also figured *O. claytoniana* consisting of four cells without a rhizoid. The early appearance of the rhizoid is an indication of the polarity characteristic of most fern gametophytes but lacking in the Hymenophyllaceae. The writer has found young gametophytes with belated rhizoid development in both the species just mentioned, and also in *O. javanica* and *Todea barbara*. In cultures of *Marattia sambucina*, a fern of the deep rain forests of Java, the writer has occasionally found prothalli with five or six cells and no rhizoid. Delay in the development of the first rhizoid, rare in the more advanced families of ferns, is apparently not unusual in the Marattiaceae and the Osmundaceae. In subsequent development, however, the gametophytes of the Hymenophyllaceae have very little in common with those of the two families mentioned. Whatever the ancestors of the Hymenophyllaceae may be, we must assume that in the evolution of the type found ordinarily in *Hymenophyllum* there has been a great reduction in the thickness of the thallus, an elimination of the midrib, and an intensification of a slight tendency to lobe or branch found in many fern gametophytes. These changes may be related to growth in weak light and a moist environment, where filmy ferns are ordinarily found. Delay in the appearance of the rhizoid may be correlated with the hygrophilous habit of the family, although in the filamentous species of *Trichomanes* (*T. auriculatum*) the rhizoid may develop earlier than in *Hymenophyl-*

*lum*. This may be related to the shortening of the length of life of the gametophyte, which is indicated by the earlier maturity of *T. auriculatum*.

The relative uniformity of the early stages of the gametophyte of *Hymenophyllum* is followed by a period of greater variability and plasticity, but the mature stages again seem to be fairly constant in type. The ribbon-like expansions show variations in details of branching, but in general habit conform to a well defined pattern. The filamentous type with its modifications is, according to our present knowledge, not common in the genus. We have no information about its behavior at maturity. It is not known whether in any species of *Hymenophyllum* the filament comes to maturity and produces sex organs, as in the filamentous species of *Trichomanes*, or whether —as in most families of ferns—it is limited to the vegetative, or at most the antheridial, stage. At present it is known only in early developmental stages of *H. javanicum* and *H. fuscum*, and from the account by METTENIUS of older stages of *H. microcarpum* which had not yet produced sex organs. Apparently there has been a reduction from the plate to the filament in *Hymenophyllum* as well as in *Trichomanes*. This has probably occurred several times in the family, and, so far as now known, there is no reason for assuming that any close relationship links the forms in which it occurs. There is apparently considerable variation in the length of time required by different species to attain maturity. We do not know how constant this is, or whether it can be correlated with the phylogenetic group, or with the ecological habit.

The developmental history of the gametophyte is known for only a few species of *Trichomanes*. As there is apparently more variation in this genus than in *Hymenophyllum*, it is highly desirable that knowledge of the development of the gametophyte should be extended to cover many more species. Information is so fragmentary that we do not know how common is the 3-celled type of germination, or its relation to the ribbon-like thallus or the mixed type in which both filaments and expansions are found. We do not know whether the pure filamentous type of gametophyte is correlated with the 4-celled type of germination, as seems probable; or even whether there is a pure filamentous type, although that seems to be the case in *T.*

*auriculatum*,[1] *T. bilabiatum*, *T. maximum*, and probably in a majority of the species of *Trichomanes*. However, as is the case of *T. sinuosum* (**14**), the production of sex organs by the filamentous stage does not preclude the possibility of development of a plate.

A comparison of the structure of the gametophyte of some of the oriental species of *Trichomanes* with the position of the species in COPELAND'S classification may be of interest, not because it affords adequate basis for any conclusions but because of suggestions of lines of investigation. Germination stages are known for representatives of only six of COPELAND'S fifteen groups. Certain points, however, seem to be worth noting. *Cardiomanes reniforme* (Forst.) Pr. (*Trichomanes reniforme* Forst.) agrees with *Hymenophyllum* in germination and in the early formation of a plate stage; the mature stage is not known. COPELAND (**7**, p. 270) says of this species, "Construing *Trichomanes* in the broadest sense, this is the only really isolated species in the Old World." In his group 1, *Pyxidifera*, both types of germination have been reported: the 3-celled type in *T. draytonianum* (**5**) and the 4-celled in *T. schmidianum* (**18, 23**). Two species have been described as filamentous at maturity: *T. pyxidifera* (**1**) and *T. colensoi* (**16**). *T. diffusum*, in group 4, *Gonocormus*, has the 4-celled type of germination (**12**). In group 5, *Microtrichomanes*, the three species for which the germination is known belong to the *Hymenophyllum* type: *T. ampliatum* (*T. digitatum* Sw.) (**18**), *T. palmatifidum* (**12**), and *T. nitidulum*. HOLLOWAY (**15**) states that *T. lyallii* has early plate development. COPELAND (**8**, p. 165) in his discussion of *Sphaerocionium*, his eighth sub-genus of *Hymenophyllum*, says, "the whole of this group, *Microtrichomanes*, as I used the term (not as PRANTL used it), belongs rather in *Hymenophyllum*, as the term is used here." *T. humile*, in group 6, *Crepidium*, has the 4-celled type of germination (**18**); and *T. bilabiatum*, of group 7, *Taschneria*, has the same type. Group 9, *Scandentia*, includes three species for which germination is known: *T. speciosum* (*T. radicans* Sw.) (**19**), *T. auriculatum*, and *T. maximum*. This group has the modified type of germination, the 4-celled type which tends to lose its sharply defined character. In germination this group seems to be farther removed from the central type than any for which there are records.

[1] When the cultures of *T. auriculatum* were two and a half years old, a few expansions three to five cells wide were found at the tips of a few filamentous branches.

Obviously much more information is needed about germination, development, and mature stages before we are in a position to estimate the value of the contribution of the gametophyte to a phylogenetic classification of the family. Even so plastic and variable a structure as the gametophyte has certain well defined characters which may be of value in the working out of groups. In a family which has presented such difficulties in classification as those noted by COPELAND, a gametophyte of so pronounced a character may well be the basis of important contributions.

## Summary

1. Germination stages are given for seven species of *Hymenophyllum: H. acanthoides, H. blumeanum, H. fuscum, H. holochilum, H. junghuhnii, H. kurzii,* and *H. javanicum.* The spores germinated while still in the sporangium with the formation of a cross wall followed by a second wall meeting the first in the center of the spore. The walls separating the three cells lie under the tripartite ridges of the spore coat. Occasionally the second wall is delayed until after discharge of the spores, or it may be suppressed.

2. After rupture of the spore coat the young gametophyte assumes the form of a triangular cushion, with the three tips growing in the direction of the longitudinal axes of the valves of the spore coat.

3. Secondary walls arise in sequence, cutting off a small cell from each tip. Development is usually symmetrical up to the 6-celled stage, but the next stages are more variable. In *H. acanthoides, H. blumeanum, H. holochilum,* and *H. kurzii* growth of one tip results in a plate; growth of the other two is checked sooner or later by rhizoid formation. The prothallus develops as a branching ribbon-like structure, which at one year may have branches 9–12 mm. long. In *H. fuscum* and *H. javanicum* growth is much slower; the later growth is filamentous in form with occasional longitudinal divisions but without plate formation.

4. Rhizoid development is belated in *Hymenophyllum;* the first rhizoid usually appears when there are six to eight cells in the gametophyte. The rhizoids have heavy brown walls; they may arise from any cell of a filament, but arise only from marginal cells of the ribbon-like thallus. Branching rhizoids are common, particularly near the

base of the gametophyte; frequently two rhizoids grow from one cell.

5. The gametophytes of *H. holochilum* and *H. kurzii* reached maturity in twenty months, bearing both antheridia and archegonia. *H. blumeanum* did not produce archegonia until nearly four years old.

6. Germination in *Trichomanes nitidulum* is like that in *Hymenophyllum*, with the spore dividing into three cells. Subsequent stages were less regular than in *Hymenophyllum* and there was no plate formation. The gametophytes remained filamentous, with longitudinal divisions in a few cells or sections of a filament.

7. In *T. auriculatum* and *T. bilabiatum*, after rupture of the spore coat and before any divisions have occurred, the spore assumes the form of a triangular cushion. The first walls cut off the three tips, leaving an undivided central cell. If growth is slow, as in the case of spores which fall into the indusium and germinate there, the 4-celled stage is symmetrical; but if conditions for growth are favorable, the form is more irregular, particularly in *T. auriculatum* in which the first tip cut off usually forms a rhizoid in which growth is relatively rapid. The wall cutting off the third tip is frequently delayed, especially in *T. auriculatum*.

8. Germination in *T. maximum* may conform to the 4-celled type, but it is usually less regular. There is a tendency toward suppression of one of the tips, or sometimes merely a delay in wall formation in one tip. The gametophyte may then develop as a straight or a lopsided filament, rather than as a triradiate structure. Growth and development in *T. maximum* are extremely slow.

9. The mature gametophytes of *T. auriculatum* and *T. bilabiatum* are of the branching filamentous type without longitudinal divisions, forming a "turf" in compact cultures. They attained maturity, producing antheridia and archegonia, in nine to ten months. The gametophyte of *T. maximum*, although developing much more slowly, was similar in form and habit. Antheridia were formed on gametophytes one year old.

10. A variation from the usual type of germination in *Hymenophyllum* was found occasionally in several species. It suggests how the two types of germination in the family may be related. Reasons

are given for looking upon the *Hymenophyllum* type as the older. Gametophytes of *Hymenophyllum* are found occasionally in which only one primary wall is formed at germination; the second is suppressed. The 4-celled type found in most species of *Trichomanes* might have arisen from the type found in *Hymenophyllum* (and a few species of *Trichomanes*) by the suppression of both of the primary walls, leaving the central part of the spore undivided. In the latter type of germination the first walls to appear in the spore correspond to the secondary walls which appear in the tips of the triangular gametophyte of *Hymenophyllum*.

The writer expresses her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for providing the excellent facilities which she enjoyed for the collection and culture of fern material. In particular she is grateful to Dr. P. DAKKUS, Mr. M. L. BRUGGEMAN, and Miss E. J. REIJKEBUSCH of Buitenzorg and Mr. C. VAN WOERDEN of Tjibodas; to Professor S. HIBINO of the University of Taihoku for arranging facilities for collecting ferns in Formosa; to Dr. LENETTE R. ATKINSON for assistance with drawings and records of cultures; to the late Professor KUDO of Taihoku, for the determination of the species of Formosan ferns; and to Dr. O. POSTHUMUS for the determination of the species of the Hymenophyllaceae collected in Java.

MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MASSACHUSETTS

## LITERATURE CITED

1. BOWER, F. O., On the normal and abnormal development of the oophyte in *Trichomanes*. Ann. Bot. **1**:269–305. 1888.
2. ———, On apospory and production of gemmae in *Trichomanes kaulfussii* Hk. and Grev. Ann. Bot. **8**:465–468. 1894.
3. ———, The ferns. Vol. II. 1926.
4. CAMPBELL, D. H., On the prothallium and embryo of *Osmunda claytoniana* L. and *Osmunda cinnamomea* L. Ann. Bot. **6**:49–94. 1892.
5. ———, Mosses and ferns. 3d ed. 1918.
6. CHRISTENSEN, C., Filicinae, in Manual of pteridology, ed. by Fr. Verdoorn. The Hague. 1938.

base of the gametophyte; frequently two rhizoids grow from one cell.

5. The gametophytes of *H. holochilum* and *H. kurzii* reached maturity in twenty months, bearing both antheridia and archegonia. *H. blumeanum* did not produce archegonia until nearly four years old.

6. Germination in *Trichomanes nitidulum* is like that in *Hymenophyllum*, with the spore dividing into three cells. Subsequent stages were less regular than in *Hymenophyllum* and there was no plate formation. The gametophytes remained filamentous, with longitudinal divisions in a few cells or sections of a filament.

7. In *T. auriculatum* and *T. bilabiatum*, after rupture of the spore coat and before any divisions have occurred, the spore assumes the form of a triangular cushion. The first walls cut off the three tips, leaving an undivided central cell. If growth is slow, as in the case of spores which fall into the indusium and germinate there, the 4-celled stage is symmetrical; but if conditions for growth are favorable, the form is more irregular, particularly in *T. auriculatum* in which the first tip cut off usually forms a rhizoid in which growth is relatively rapid. The wall cutting off the third tip is frequently delayed, especially in *T. auriculatum*.

8. Germination in *T. maximum* may conform to the 4-celled type, but it is usually less regular. There is a tendency toward suppression of one of the tips, or sometimes merely a delay in wall formation in one tip. The gametophyte may then develop as a straight or a lopsided filament, rather than as a triradiate structure. Growth and development in *T. maximum* are extremely slow.

9. The mature gametophytes of *T. auriculatum* and *T. bilabiatum* are of the branching filamentous type without longitudinal divisions, forming a "turf" in compact cultures. They attained maturity, producing antheridia and archegonia, in nine to ten months. The gametophyte of *T. maximum*, although developing much more slowly, was similar in form and habit. Antheridia were formed on gametophytes one year old.

10. A variation from the usual type of germination in *Hymenophyllum* was found occasionally in several species. It suggests how the two types of germination in the family may be related. Reasons

are given for looking upon the *Hymenophyllum* type as the older. Gametophytes of *Hymenophyllum* are found occasionally in which only one primary wall is formed at germination; the second is suppressed. The 4-celled type found in most species of *Trichomanes* might have arisen from the type found in *Hymenophyllum* (and a few species of *Trichomanes*) by the suppression of both of the primary walls, leaving the central part of the spore undivided. In the latter type of germination the first walls to appear in the spore correspond to the secondary walls which appear in the tips of the triangular gametophyte of *Hymenophyllum*.

The writer expresses her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for providing the excellent facilities which she enjoyed for the collection and culture of fern material. In particular she is grateful to Dr. P. DAKKUS, Mr. M. L. BRUGGEMAN, and Miss E. J. REIJKEBUSCH of Buitenzorg and Mr. C. VAN WOERDEN of Tjibodas; to Professor S. HIBINO of the University of Taihoku for arranging facilities for collecting ferns in Formosa; to Dr. LENETTE R. ATKINSON for assistance with drawings and records of cultures; to the late Professor KUDO of Taihoku, for the determination of the species of Formosan ferns; and to Dr. O. POSTHUMUS for the determination of the species of the Hymenophyllaceae collected in Java.

MOUNT HOLYOKE COLLEGE
SOUTH HADLEY, MASSACHUSETTS

## LITERATURE CITED

1. BOWER, F. O., On the normal and abnormal development of the oophyte in *Trichomanes*. Ann. Bot. 1:269–305. 1888.
2. ———, On apospory and production of gemmae in *Trichomanes kaulfussii* Hk. and Grev. Ann. Bot. 8:465–468. 1894.
3. ———, The ferns. Vol. II. 1926.
4. CAMPBELL, D. H., On the prothallium and embryo of *Osmunda claytoniana* L. and *Osmunda cinnamomea* L. Ann. Bot. 6:49–94. 1892.
5. ———, Mosses and ferns. 3d ed. 1918.
6. CHRISTENSEN, C., Filicinae, in Manual of pteridology, ed. by Fr. Verdoorn. The Hague. 1938.

7. COPELAND, E. B., *Trichomanes*. Phil. Jour. Sci. **51**:119–280. 1933.
8. ———, *Hymenophyllum*. Phil. Jour. Sci. **64**:1–188. 1937.
9. GEORGEVITCH, PETER, Preliminary note on apospory and apogamy in *Trichomanes kaulfussii* Hk. and Grev. Ann. Bot. **24**:233–234. 1910.
10. ———, Aposporie und Apogamie bei *Trichomanes kaulfussii* Hk. and Grev. Jahrb. Wiss. Bot. **48**:155–170. 1910.
11. GIESENHAGEN, K., Die Hymenophyllaceen. Flora **73**:411–464. 1890.
12. GOEBEL, K., Zur Keimungsgeschichte einiger Farne. Ann. Jard. Bot. Buitenzorg **7**:74–119. 1887.
13. ———, Archegonienstudien über die Geschlechtsgeneration der Hymenophyllaceae. Flora **76**:92–116. 1892.
14. ———, Organographie der Pflanzen. Teil 2. 3d. ed. 1930.
15. HOLLOWAY, J. E., Studies in the New Zealand Hymenophyllaceae. Part I. Trans. New Zealand Inst. **54**:577–618. 1923.
16. ———, The experimental cultivation of the gametophytes of *Hymenophyllum pulcherrimum* Col. and *Trichomanes reniforme* Forst. Ann. Bot. **44**:269–284. 1930.
17. JANCZEWSKI, ED., and ROSTAFINSKI, J., Note sur la prothalle de l'*Hymenophyllum tunbridgense*. Mém. Soc. Nat. Sci. Natur. Cherbourg. 1875. Rev. in Just's Bot. Jahrb. **3**:328, 331, 333. 1877.
18. METTENIUS, G., Ueber die Hymenophyllaceen. Abhandl. Math-Phys. Klasse Königl. Sächs. Ges. Wiss. **7**:401–501. 1864.
19. PRANTL, K., Untersuchungen zur Morphologie der Gefässkryptogamen. I. Die Hymenophyllaceen. Leipzig. 1875.
20. PRESL, K. B., Hymenophyllaceae. 1843.
21. SADEBECK, R., Hymenophyllaceae in ENGLER and PRANTL's Nat. Pflanzenfamilien. Teil I, Abt. 4. 1902.
22. STOKEY, ALMA G., The prothallia of the Cyatheaceae. BOT. GAZ. **90**:1–45. 1930.
23. TASCHNER, C. F., Dissertatio: De duabus novis *Trichomanum* speciebus de earum nec non aliarum huis generis plantarum structure. Jena. 1843.

# ABSORPTION OF SOIL MOISTURE BY MAIZE ROOTS

CHARLES HOMER DAVIS

(WITH THREE FIGURES)

## Introduction

Little is known of the mechanics of water absorption from the soil by the roots of plants. ROSENE (**7**) has recently summarized the data on water absorption by different root zones. WEAVER (**8**) found that root hairs soon died in dry soil. POPESCO (**5**) noted that dead root hairs could be active in absorption. ALDRICH, WORK, and LEWIS (**1**) found a high correlation between the concentration of fine roots and the amount of water absorbed in the previous season. They observed that the growth of pear fruits stopped well before the soil water was reduced to the wilting coefficient, and also that the leaves wilted before the wilting point of the soil moisture was reached. LOOMIS (**4**) observed that maize plants stopped growth under conditions of high transpiration although the moisture present in the soil was nearly optimum. ALDRICH, WORK, and LEWIS pointed out the possibility that wilting of the leaves of pear trees may have been caused by the drying of the soil below the wilting point within a short distance of the small roots, while large samples necessary for moisture determinations did not show a critically low moisture content. The present investigation, started in 1937, was designed to determine the absorption of water from soil in which root development could be observed.

## Investigation

### RELATION OF ROOT POSITION TO MOISTURE ABSORPTION

Maize was planted at one end of a wooden box in which one side had been replaced with glass, through which the roots could be observed as they grew outward and downward from the plant (fig. 1). Rate of growth and distribution of the roots were studied daily by removing the black cardboard, which covered the glass side, and observing the roots which could be seen through the glass. Moisture absorption was measured by daily readings of RICHARDS' soil-mois-

ture tensiometers (6) set at 4-inch intervals in the box, 6 inches below the surface and halfway between the back and front of the box. Tensiometers measure the surface tension of the water directly with a mercury manometer, which is connected to the soil moisture through the walls of a porous clay cup by an air-free water system. Since the mercury is in a capillary tube and its weight resists the loss

FIG. 1.—Box used in the greenhouse at Ames, showing arrangement of plants and tensiometers.

of water to the soil, only a small quantity of water is lost to the soil from the tensiometer. The mercury manometer is dependent upon air pressure, so the limit of tension measured by the tensiometer is one atmosphere. The instruments used in these experiments failed to record accurately above 600 mm. of mercury. Soil moisture percentages below the range of the tensiometers were determined by sampling with a sharpened steel tube.

Figures 2 and 3 give the moisture levels, reciprocal of tension reading, for the alternate days during two drying periods. During the first period the roots had extended as shown at the top of figure 2

on the dates indicated. The roots reached the end of the box April 6 and thereafter appeared to be uniformly distributed throughout the

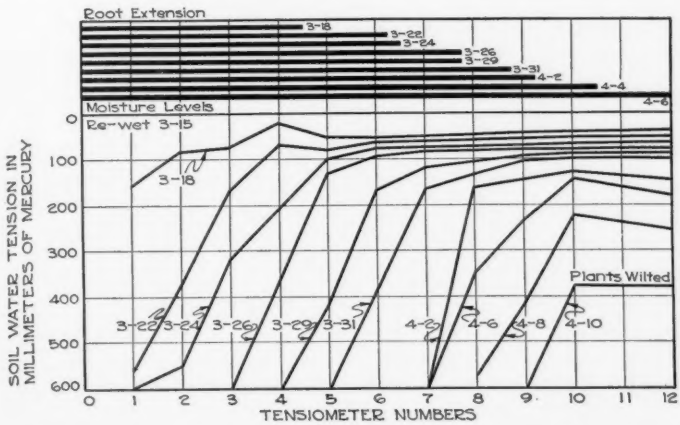



FIG. 2.—Root extension (top) and moisture absorption tensions at distances of 4-48 inches from group of corn plants.

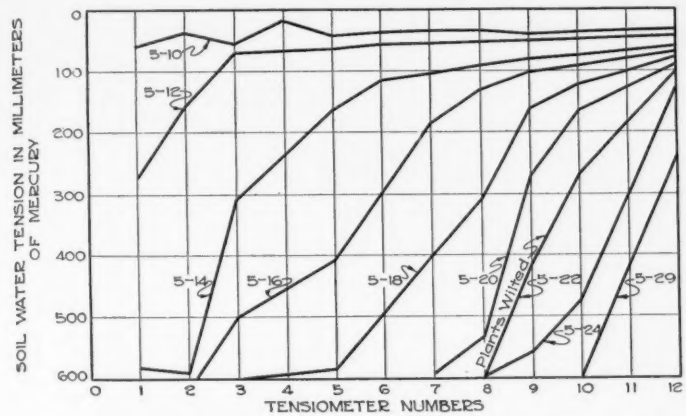



FIG. 3.—Moisture absorption tensions in a box uniformly filled with roots from plants at the left. Tensiometers spaced 4 inches apart.

soil. The soil was watered on March 15 and seven days later the soil near the plants had been dried almost to the limit of the tensiom-

eter range. At the same time the roots had extended past tensiometer no. 6, 24 inches from the plant, but appreciable moisture absorption took place no farther than 12 inches from the plant, as shown by the rise in tensiometer no. 3. Progressive absorption is shown for the successive periods. On March 31, when the roots were 7 inches beyond tensiometer no. 7, appreciable moisture absorption was taking place near that tensiometer. Two days later the soil near no. 7 was too dry to measure but appreciable moisture absorption was taking place near tensiometer no. 8. Roots had extended 6 inches past no. 9 on April 2 but that tensiometer did not show appreciable water absorption from the soil until April 6, when the roots had reached the end of the box. The slow increase in tension shown by the tensiometers not in the root area was probably due to air drying, although the soil was covered with a 3:2 paraffin-vaseline mixture to reduce this loss. On April 10 roots were established in moist soil in the end of the box 4 feet from the plants, yet the plants were permanently wilted under greenhouse conditions.

The wilting percentage of the soil was determined with maize from the same seed lot. The average of twenty-five determinations was 9.58 per cent, and the range was from 8.28 when the plants in a pot were severely wilted to 11.60 per cent soil moisture in a pot in which the plants were barely wilted but remained wilted overnight in a moist chamber. The soil in the box on April 16, as shown in table 1, was below the determined wilting percentage 2 feet from the plants and the plants remained wilted overnight; yet 3 feet from the plants the soil moisture was above the wilting percentage and half the soil available to the root system contained available moisture.

The soil was then re-wet and the moisture was again absorbed, first near the plants and later farther from them. Root hairs were more numerous at all times on the root tips farther from the plants, and in relatively moist soil, than on the roots near the plants in soil which was dried more frequently. All the roots had many small branches, on a few of which root hairs developed. Those fine roots with hairs which appeared on the glass front soon lost their hairs, and root hairs were never observed on many of the small rootlets.

After April 26 the soil as seen through the glass front was uniformly filled with roots and the moisture absorptions shown in ta-

TABLE 1

MOISTURE PERCENTAGES BY DATE AND POSITION, AND NUMBERS OF ROOTS APPEARING AGAINST EACH 4 INCHES OF GLASS FRONT OF THE BOX EXPERIMENT AT AMES. WILTING PERCENTAGE OF SOIL, 9.6

| DATE | POSITION OF SAMPLES AND ROOTS IN INCHES FROM PLANTS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | INCHES DEPTH | NOTES |
| March 11 | ... | ... | ... | 14 | ... | ... | ... | ... | 15 | ... | ... | 1–3 | Plants wilted |
| | ... | ... | ... | 13 | ... | ... | ... | ... | 16 | ... | ... | 3–6 | |
| | ... | ... | ... | 14 | ... | ... | ... | ... | 14 | ... | ... | 6–10 | |
| No. roots | 16 | 21 | 14 | 9 | ... | ... | ... | ... | ... | ... | ... | ..... | |
| March 12 | ... | 19 | ... | ... | ... | ... | ... | ... | 19 | ... | ... | 1–3 | Re-wet after sampling |
| | ... | 15 | ... | ... | ... | ... | ... | ... | 15 | ... | ... | 3–6 | |
| | ... | 12 | ... | ... | ... | ... | ... | ... | 15 | ... | ... | 6–10 | |
| No. roots | 16 | 21 | 16 | 9 | ... | ... | ... | ... | ... | ... | ... | ..... | |
| March 17 | ... | ... | 21 | ... | ... | ... | ... | ... | ... | 25 | ... | 1–3 | |
| | ... | ... | 23 | ... | ... | ... | ... | ... | ... | 23 | ... | 3–6 | |
| | ... | ... | 20 | ... | ... | ... | ... | ... | ... | 26 | ... | 6–10 | |
| No. roots | 16 | 21 | 16 | 14 | ... | ... | ... | ... | ... | ... | ... | ..... | |
| March 29 | ... | 13 | ... | ... | ... | 19 | ... | ... | ... | 25 | ... | 1–3 | |
| | ... | 11 | ... | ... | ... | 19 | ... | ... | ... | 22 | ... | 3–6 | Re-wet April 10 |
| | ... | 10 | ... | ... | ... | 19 | ... | ... | ... | 22 | ... | 6–10 | |
| No. roots | 16 | 22 | 16 | 19 | 5 | 9 | 5 | 2 | 1 | ... | ... | ..... | |
| April 16 | ... | ... | ... | 8 | ... | 8 | ... | ... | 13 | ... | ... | 1–3 | Re-wet after sampling |
| | ... | ... | ... | 8 | ... | 7 | ... | ... | 11 | ... | ... | 3–6 | |
| | ... | ... | ... | 7 | ... | 8 | ... | ... | 9 | ... | ... | 6–10 | |
| No. roots | 16 | 22 | 16 | 23 | 14 | 23 | 16 | 9 | 7 | 2 | 2 | ..... | |
| April 18 | ... | ... | ... | 25 | ... | ... | 30 | ... | 27 | ... | ... | 1–3 | |
| | ... | ... | ... | 21 | ... | ... | 23 | ... | 24 | ... | ... | 3–6 | |
| | ... | ... | ... | 9 | ... | ... | 22 | ... | 22 | ... | ... | 6–10 | |
| No. roots | 16 | 22 | 16 | 23 | 14 | 23 | 16 | 12 | 7 | 5 | 2 | ..... | |
| April 26 | 10 | ... | ... | 13 | ... | ... | 15 | ... | 19 | ... | ... | 1–3 | |
| | 10 | ... | ... | 11 | ... | ... | 14 | ... | 17 | ... | ... | 3–6 | Wilted May 5 |
| | 10 | ... | ... | 10 | ... | ... | 14 | ... | 16 | ... | ... | 6–10 | |
| No. roots | 16 | 22 | 16 | 23 | 14 | 23 | 16 | 14 | 13 | 17 | 14 | ..... | |
| May 7 | ... | ... | 7 | ... | ... | ... | 8 | ... | ... | 11 | ... | 1–3 | Re-wet after sampling |
| | ... | ... | 7 | ... | ... | ... | 8 | ... | ... | 9 | ... | 3–6 | |
| | ... | ... | 7 | ... | ... | ... | 7 | ... | ... | 9 | ... | 6–10 | |

TABLE 1—*Continued*

| DATE | POSITION OF SAMPLES AND ROOTS IN INCHES FROM PLANTS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | INCHES DEPTH | NOTES |
| May 10 | ... | ... | 22 | ... | ... | ... | 21 | ... | ... | 22 | ... | 1–3 | |
| | ... | ... | 22 | ... | ... | ... | 22 | ... | ... | 23 | ... | 3–6 | |
| | ... | ... | 23 | ... | ... | ... | 23 | ... | ... | 24 | ... | 6–10 | |
| May 15 | 9 | ... | ... | 13 | ... | ... | ... | 18 | ... | ... | ... | 1–3 | |
| | 9 | ... | ... | 13 | ... | ... | ... | 18 | ... | ... | ... | 3–6 | |
| | 9 | ... | ... | 11 | ... | ... | ... | 19 | ... | ... | ... | 6–10 | |
| May 22 | ... | 8 | ... | ... | ... | ... | ... | ... | 14 | ... | 18 | 1–3 | Wilted at noon |
| | ... | 8 | ... | ... | ... | ... | ... | ... | 13 | ... | 18 | 3–6 | |
| | ... | 8 | ... | ... | ... | ... | ... | ... | 12 | ... | 18 | 6–10 | |
| May 29 | ... | ... | 7 | ... | ... | ... | 8 | ... | ... | ... | 16 | 1–3 | Re-wet after sampling |
| | ... | ... | 8 | ... | ... | ... | 9 | ... | ... | ... | 16 | 3–6 | |
| | ... | ... | 7 | ... | ... | ... | 8 | ... | ... | ... | 15 | 6–10 | |
| June 8 | 10 | ... | 16 | ... | ... | ... | ... | ... | 19 | ... | ... | 1–3 | |
| | 10 | ... | 14 | ... | ... | ... | ... | ... | 20 | ... | ... | 3–6 | |
| | 9 | ... | 14 | ... | ... | ... | ... | ... | 20 | ... | ... | 6–10 | |

ble 1 after April 26 and in figure 3 represent absorption at different rates by apparently equal numbers of roots. Between May 10 and 14 the soil near tensiometer no. 1 was dried by water absorption to a moisture percentage below the wilting percentage, while 20 inches from the plants only a small amount of water had been absorbed. Six days later, on May 20, the soil near tensiometer no. 8, 32 inches from the plant, was almost as dry as the lower moisture range of the tensiometer, but only small amounts of water were being absorbed near no. 12. The plants wilted at noon on May 22, two days later, when the soil moisture as near the plant as no. 9 and no. 10 (36 inches away) was still above the wilting percentage, and the soil moisture near no. 11 was only 2 per cent below the field capacity of the soil. Moisture near the plants was reduced to the wilting percentage on May 15, seven days before the plants showed any tendency to wilt. The box was re-wet after May 29 and on June 8 the soil near the plants was already near the wilting percentage, while 3 feet away from the plants moisture in the soil was near the field capacity.

The soil used at Ames was Clarion silt loam. Later the same ex-

periments were repeated at Tucson, Arizona, with Gila silt loam. The experiments at Tucson differed in that tensiometers were not used; one plant instead of five was left in the box; the roots were counted more accurately to include all the fine roots, and the box was slightly longer (53 inches). In the absence of tensiometers, moisture samples were taken more regularly. The percentages of moisture found are shown in table 2. The two samples for a given location in a single day were taken from the surface to 6 inches deep and from 6 inches to the bottom of the box. The observed wilting percentage of the soil used at Tucson was 6.76. On November 29 the plant wilted at noon. The percentages of soil moisture near the plant were near the wilting percentage, but 20 inches from the plant water was readily available. The plant recovered overnight. On December 7 the plant wilted at 10 A.M., when the moisture samples were taken (table 2). After the low values of 3 and 5 per cent were found, samples were taken again on December 8 to check these values. The second samples, 8 inches farther from the plant, contained only 4 and 5 per cent moisture and show that the roots near the plant absorbed water well below the determined wilting percentage, while roots farther from the plant were still in soil moistened above the wilting percentage. The plant did not recover turgor after wilting on December 7 until the soil was re-wet on December 8.

From December 8 to 21, roots 32 inches from the plant and ranging in number from sixteen to seventy absorbed water down to 13 and 11 per cent soil moisture. During the same period twenty-nine to thirty-three roots 8 inches from the plant absorbed water down to 7 and 8 per cent soil moisture, or about the wilting percentage. After each wetting the same greater absorption near the plant was observed, although similar numbers of roots were present farther from the plant.

The box was thoroughly saturated on December 24 and by December 27 the soil was dry enough to sample. On January 2 appreciable quantities of moisture had been absorbed near the plant, while 24 inches away the soil moisture was near the field percentage. After the samples of January 2 were taken, the soil was cut into 3-inch sections and the roots appearing in the cross sections were counted. The numbers of roots and the average of the numbers of roots of the

TABLE 2

MOISTURE PERCENTAGES AND ROOT DISTRIBUTION BY 4-INCH INTERVALS
EXPERIMENT AT TUCSON. WILTING PERCENTAGE, 6.8

| DATE | DISTANCE FROM PLANT IN INCHES | | | | | | | | | | | | | NOTES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 53 | |
| November 4 | ... | 5 | ... | ... | 8 | ... | ... | ... | 10 | ... | ... | ... | ... | Re-wet after sampling |
| | ... | 8 | ... | ... | 8 | ... | ... | ... | 12 | ... | ... | ... | ... | |
| No. roots | ... | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | |
| November 10 | ... | ... | 25 | 23 | 22 | ... | ... | ... | ... | 22 | ... | ... | ... | |
| | ... | ... | 29 | 17 | 23 | ... | ... | ... | ... | 25 | ... | ... | ... | |
| No. roots | ... | 3 | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | |
| November 19 | ... | 8 | ... | 9 | ... | ... | 14 | ... | ... | 15 | ... | ... | ... | |
| | ... | 8 | ... | 9 | ... | ... | 15 | ... | ... | 15 | ... | ... | ... | |
| No. roots | 5 | 3 | 10 | 5 | 3 | 2 | ... | ... | ... | ... | ... | ... | ... | |
| November 21 | ... | ... | 6 | ... | ... | 10 | ... | 11 | ... | ... | 12 | ... | ... | |
| | ... | ... | 7 | ... | ... | 9 | ... | 13 | ... | ... | 15 | ... | ... | |
| No. roots | 8 | 14 | 16 | 21 | 17 | 12 | 9 | ... | ... | ... | ... | ... | ... | |
| November 29 | ... | 7 | ... | ... | 13 | ... | ... | 16 | ... | ... | 19 | ... | ... | Plant wilted at noon |
| | ... | 8 | ... | ... | 9 | ... | ... | 17 | ... | ... | 22 | ... | ... | |
| No. roots | 12 | 23 | 33 | 52 | 50 | 52 | 24 | 9 | ... | ... | ... | ... | ... | |
| December 7 | 3 | ... | ... | ... | ... | 6 | ... | 10 | 12 | ... | ... | ... | ... | Wilted at 10 A.M. |
| | 5 | ... | ... | ... | ... | 6 | ... | 10 | 15 | ... | ... | ... | ... | |
| No. roots | 19 | 29 | 31 | 53 | 52 | 51 | 20 | 17 | 4 | ... | ... | ... | ... | |
| December 8 | ... | ... | 4 | ... | ... | ... | 6 | ... | ... | ... | ... | ... | ... | Wilted, re-wet |
| | ... | ... | 5 | ... | ... | ... | 7 | ... | ... | ... | ... | ... | ... | |
| December 18 | ... | ... | 10 | ... | 13 | ... | ... | ... | ... | 16 | ... | 19 | ... | |
| | ... | ... | 10 | ... | 11 | ... | ... | ... | ... | 19 | ... | 19 | ... | |
| No. roots | 28 | 33 | 37 | 65 | 83 | 69 | 32 | 56 | 43 | 28 | 15 | ... | ... | |
| December 21 | ... | 7 | ... | ... | 10 | ... | ... | 13 | ... | ... | 16 | ... | 17 | |
| | ... | 8 | ... | ... | 8 | ... | ... | 11 | ... | ... | 18 | ... | 19 | |
| No. roots | 40 | 33 | 51 | 71 | 86 | 77 | 33 | 70 | 66 | 54 | 39 | 3 | ... | |
| December 24 | ... | ... | ... | 6 | ... | 8 | ... | ... | ... | ... | 13 | 13 | ... | Wilted, re-wet |
| | ... | ... | ... | 6 | ... | 7 | ... | ... | ... | ... | 12 | 15 | ... | |
| No. roots | 36 | 34 | 48 | 58 | 73 | 58 | 36 | 69 | 53 | 59 | 47 | 10 | ... | |

TABLE 2—*Continued*

| Date | Distance from plant in inches | | | | | | | | | | | | | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 53 | |
| December 27 | 23 | ... | 30 | ... | ... | ... | ... | 29 | ... | ... | ... | 34 | ... | Soil still flooded |
| | 24 | ... | 26 | ... | ... | ... | ... | 30 | ... | ... | ... | 34 | ... | |
| No. roots | 41 | 36 | 49 | 57 | 75 | 60 | 39 | 64 | 64 | 68 | 52 | 28 | 30 | |
| January 1 | ... | 17 | ... | 19 | ... | ... | ... | 19 | ... | 26 | ... | ... | 25 | |
| | ... | 16 | ... | 19 | ... | ... | ... | 21 | ... | 25 | ... | ... | 26 | |
| No. roots | 37 | 33 | 45 | 61 | 80 | 64 | 39 | 50 | 60 | 71 | 70 | 52 | 28 | |
| January 2 | 11 | ... | 16 | ... | 17 | 22 | ... | 22 | ... | 20 | 22 | 24 | 25 | |
| | 15 | ... | 17 | ... | ... | 20 | ... | 23 | ... | ... | 25 | 25 | ... | |

three adjacent 1×10-inch areas on the glass front are recorded in table 3. The correlation coefficient of 0.81 is positive and significant, showing that the counts made on the glass front were a reasonably good estimate of the total numbers of roots in the box.

TABLE 3

CORRELATION BETWEEN ROOTS IN CROSS SECTION OF SOIL AND IN ADJACENT SURFACE EXPOSED THROUGH A GLASS WALL

| Area | Inches from plant | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | |
| Roots in soil section | 54 | 50 | 47 | 41 | 49 | 58 | 67 | 56 | 62 | 48 | 66 | 60 | 64 | 62 | 59 | 40 | |
| Roots in glass front | 9 | 14 | 12 | 11 | 14 | 17 | 20 | 14 | 10 | 15 | 13 | 16 | 20 | 21 | 14 | 9 | |
| | Correlation..... $r=+0.81$ | | | | | | | | | | | | | | | | |

## DEPENDENCE OF GROWTH UPON SOIL MOISTURE

The effect of moisture supply on the growth of young maize plants was studied in 2-gallon pots in which the factor of distance between plant and soil moisture was minimized. When the plants were approximately 40 mm. high they were thinned to ten in each of four pots. The percentages of moisture in the soil were calculated from

the gross weights of the pots and wax seal, and the known weights of the dry soil they contained. Growth of the plants was estimated by the total increases in height of the tallest leaves on all the plants in a pot and calculated as growth in millimeters per pot per hour. During the 41 days of growth from which the data of table 4 were calculated there was no evidence of a logarithmic growth rate. The pronounced effects on growth rate caused by the fluctuations in soil moisture completely obscured any logarithmic relationships.

The first line of table 4 shows that the total growth in the 24 hours just preceding the measurement on pot 1 averaged 0.88 mm. an hour; at the time of making the growth measurements the pot was weighed, and the soil moisture percentage calculated from the gross weight was 16.96. During the next period the ten plants grew at the rate of 1.52 mm. per hour and the soil moisture percentage at the end of the period had dropped to 14.61. During the third period the growth rate was reduced to 0.37 mm. per hour and the soil moisture to 10.48 per cent. During the next period the growth was negative, a loss of 6 mm. in twenty hours, and the soil moisture was reduced to 9.63 per cent. Negative growths were caused by necrosis of the tender tips as the soil became dry. After the plants had wilted or stopped growth the pots were re-wet to the capillary capacity of the soil and the measurements continued. In many cases several days were required for the young growing leaf to reach the height attained by an older leaf which had stopped growth during the dry days. These tardy tips just after wetting resulted in lower rates of increase than would have resulted from measurements of the growing tip. The intervals between measurements were not so regular as desired and in some cases the rates of increase were calculated for as long as three days. The final moisture percentages after the longer periods are too low an estimate of the soil moisture conditions under which growth took place. Negative growths were not used in the calculation of the correlation coefficient because they were influenced greatly by a single tender tip at the time the pot became dry. The correlation coefficient of 0.53 is positive and significant, and includes discrepancies in time of measurement and soil moisture determinations, which usually were too low, and excludes negative rates of growth. The numerical value of the coefficient, but not the significance, could be increased by ex-

## TABLE 4

CORRELATION OF SOIL MOISTURE PERCENTAGES WITH TOTAL INCREASES IN PLANT HEIGHT IN MILLIMETERS PER POT PER HOUR MEASUREMENTS WHICH SHOWED NO GROWTH OMITTED. WILTING PERCENTAGE OF SOIL, 6.8

| PERCENTAGE MOISTURE | RATE OF GROWTH | PERCENTAGE MOISTURE | RATE OF GROWTH |
|---|---|---|---|
| POT I | | POT III | |
| 16.96 | 0.88 | 15.86 | 1.04 |
| 14.61 | 1.52 | 13.21 | 0.87 |
| 10.48 | 0.37 | 10.92 | 1.78 |
| 21.24 | 7.71 | 10.57 | 1.31 |
| 20.63 | 6.79 | 18.66 | 9.38 |
| 15.86 | 5.35 | 12.42 | 1.60 |
| 11.97 | 3.42 | 23.67 | 3.62 |
| 21.84 | 3.00 | 13.24 | 3.08 |
| 16.58 | 3.92 | 10.81 | 1.22 |
| 11.41 | 1.95 | 17.61 | 4.33 |
| 10.67 | 2.54 | 13.17 | 0.26 |
| 22.48 | 3.73 | 9.50 | 0.37 |
| 16.83 | 1.45 | 19.13 | 2.30 |
| 10.76 | 2.50 | 12.13 | 0.14 |
| 16.74 | 1.96 | | |
| 12.81 | 0.37 | | |
| 10.70 | 0.17 | | |
| 13.67 | 1.69 | | |
| 16.81 | 4.07 | | |
| 12.98 | 1.83 | | |
| 10.25 | 0.43 | | |
| POT II | | POT IV | |
| 19.42 | 1.37 | 17.80 | 0.87 |
| 16.73 | 0.82 | 15.09 | 1.11 |
| 11.52 | 0.46 | 10.86 | 0.27 |
| 10.37 | 0.18 | 9.74 | 0.29 |
| 23.40 | 7.29 | 23.50 | 8.21 |
| 22.65 | 4.71 | 18.01 | 5.85 |
| 17.55 | 10.30 | 13.25 | 7.79 |
| 12.99 | 6.92 | 16.38 | 2.42 |
| 17.24 | 3.40 | 11.07 | 1.91 |
| 10.93 | 3.84 | 10.73 | 2.02 |
| 10.08 | 3.41 | 23.84 | 2.78 |
| 24.45 | 2.78 | 17.83 | 2.43 |
| 18.62 | 7.62 | 11.19 | 2.17 |
| 10.70 | 2.54 | 18.33 | 4.19 |
| 18.48 | 3.19 | 13.70 | 2.26 |
| 13.34 | 2.67 | 11.20 | 0.13 |
| 10.84 | 0.09 | 18.24 | 0.67 |
| 14.71 | 1.55 | 18.41 | 6.45 |
| 18.98 | 3.72 | 14.00 | 4.00 |
| 10.24 | 0.54 | 10.67 | 0.07 |

Correlation..... $r = +0.53$

cluding some of the rates taken for longer periods and by using average soil moisture percentages, or by using percentage of available moisture rather than the directly measured soil moisture.

Under the conditions of this experiment the growth of young maize plants was influenced directly by the amount of moisture in the soil from the capillary capacity, 23 per cent, to 9.5 per cent. Plants ceased to grow or decreased in total leaf height when the soil moisture percentage was below 9.5. Table 5 shows the average moisture percentages at the ends of the periods during which growth of the plants ceased from lack of moisture.

TABLE 5

MOISTURE PERCENTAGES IN POTS AT TIME GROWTH STOPPED

| Pot I | Pot II | Pot III | Pot IV |
|---|---|---|---|
| 10.06 | 9.86 | 9.74 | 9.38 |
| 10.76 | 10.02 | 9.98 | 11.74 |
| 9.57 | 9.57 | 9.50 | 9.94 |
| 9.78 | 9.86 | 11.30 | 10.07 |

| | |
|---|---|
| Average | 9.88 |
| Wilting percentage | 6.76 |
| Average available moisture when growth stopped | 3.12 |

The pots were watered when growth stopped. When growth stopped before wilting occurred, the cessation of growth does not represent observable wilting in any of the plants during any part of the day observed. The soil moisture percentages when growth ceased ranged from 11.74 to 9.38. The average of the sixteen determinations was 9.88 per cent. The wilting percentage, determined as the moisture to which these plants were able to reduce the soil in the pots, was 6.76, and the wilting coefficient calculated from the moisture equivalent was 9.1 per cent soil moisture. Growth of the plants stopped when 3 per cent of soil moisture as measured by the wilting percentage and 0.75 per cent of soil moisture as measured by the wilting coefficient were available.

## Discussion

The selective absorption of water near the plant, when similar numbers of roots of the same plant were in soil of higher moisture content farther from the plant, indicates a moisture absorption gradient in the roots. The roots near the plant absorbed water from soil at or below the wilting percentage, while the same root or other roots 2–4 feet away were absorbing moisture at a level halfway between the wilting percentage and the field percentage. After the soil near the plant was reduced below the wilting percentage, the roots in soil moistened 4–5 per cent above the wilting percentage but 4 feet from the plant did not obtain enough water to prevent permanent wilting. There is thus no evidence for a "build up" of moisture in the soil near the plant, such as was shown by BREAZEALE (**2**), even when the roots 3–4 feet away were in soil moisture at or near the capillary capacity of the soil.

The differential rates of absorption near and away from the plant introduce a factor of distance into the wilting percentage of the soil. Should larger volumes of soil be used in determining the wilting percentage, as has been done in the field by ALDRICH, WORK, and LEWIS (**1**), BURR (**3**), and others, the field moisture percentage at wilting or maturation of a crop might be either above or below the wilting percentage determined with the same plants in a small pot. The availability of water in this experiment was not measured alone by the force with which the soil held the water, or the soil moisture percentage, but also by the distance between the moisture and the plant.

Growth of corn leaves was directly related to the amount of available moisture in the soil. Corn leaves grew less rapidly as the moisture supply was reduced. The availability of water for growth thus appeared to be less as the soil water was reduced only 2 or 3 per cent below the capillary capacity of the soil, and apparently growth no longer took place when 3 per cent of moisture was still available for the life of the plant. Plants in the large glass-front boxes did not stop growth after temporary wilting in the afternoons when sufficient moisture was available for them to regain turgor under cooler and more humid conditions; but even though moisture was available 3–4 feet from the plants, insufficient moisture was absorbed to

maintain turgor or growth when the soil near the plant was dry. The plants observed in these experiments appeared to grow more rapidly when adequate water was supplied to the leaves than when the water supply was reduced, as the moisture percentage near the plant was lowered. Measurements of ALDRICH, WORK, and LEWIS (1) on pear fruits and their observations on the leaves of the same trees indicate that the fruits and the leaves were not supplied equally with moisture as the water supply decreased. Their data on fruit growth and the observations of corn just described indicate the dynamic and complex nature of the relation between soil moisture and plant growth and demonstrate the need for more information on the problem.

## Summary

1. Observations of roots of young maize plants in a glass-front box were reasonably good estimates of the numbers of roots in a narrow box in the greenhouse.

2. Roots of established plants absorbed water from the soil more rapidly near the plant than at a distance of 3 or 4 feet. Roots of growing plants extracted water below the wilting percentage in soil near the plant when similar numbers of roots were present in moisture above the wilting percentage 4 feet away.

3. The water 4 feet from the plant was eventually absorbed after the soil near the plant was dried below the wilting percentage.

4. Growth of young plants under the conditions of these experiments was slowed by decreasing soil moisture and was stopped when the soil moisture percentage was still above the wilting percentage and the calculated wilting coefficient.

5. The absorption of water by roots is apparently a dynamic activity depending on the quantity of water in the soil and the distance between the water and the transpiring tops of the plants.

The writer wishes to acknowledge the help of Dr. W. E. LOOMIS, Iowa State College, during the performance of these experiments and in the preparation of the manuscript.

UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

## LITERATURE CITED

1. ALDRICH, W. W., WORK, R. A., and LEWIS, M. R., Pear root concentration in relation to soil moisture extraction in heavy clay soil. Jour. Agr. Res. **50**: 975–987. 1935.
2. BREAZEALE, J. F., Maintenance of moisture equilibrium and nutrition of plants at and below the wilting percentage. Arizona Agr. Exp. Sta. Tech. Bull. 29. 1930.
3. BURR, W. W., The storage and use of soil moisture. Nebraska Agr. Exp. Sta. Res. Bull. 5. 1914.
4. LOOMIS, W. E., Daily growth of maize. Amer. Jour. Bot. **21**:1–6. 1934.
5. POPESCO, ST., Recherches sur la région absorbante de la racine. Bul. Agr. Bucharest 4. No. 10. 1926.
6. RICHARDS, L. A., and GARDNER, W., Tensiometers for measuring the capillary tension of soil water. Amer. Soc. Agron. Jour. **28**:352–358. 1936.
7. ROSENE, HILDA F., Distribution of the velocities of absorption of water in the onion root. Plant Physiol. **12**:1–19. 1937.
8. WEAVER, J. E., Investigation of the root habits of plants. Amer. Jour. Bot. **12**:502–510. 1925.

# FLORAL INITIATION IN BILOXI SOYBEANS AS INFLUENCED BY AGE AND POSITION OF LEAF RECEIVING PHOTOPERIODIC TREATMENT

H. A. BORTHWICK[1] AND M. W. PARKER[2]

## Introduction

It has been demonstrated with various plants (**1, 2, 3, 4, 5**) that the photoperiodic stimulus causing initiation of flowers is received by the leaves. Young leaves of soybeans when subjected to short photoperiods have been found to be very effective in causing such initiation. This fact may be due to the age of the leaf or to its proximity to the terminal meristem. The purpose of this investigation was to determine the relative influence that these factors play in controlling photoperiodic induction.

## Material and methods

Biloxi soybeans were grown in the greenhouse on daily photoperiods of 16 or more hours until they reached the size or age desired. In certain experiments photoperiodic treatments were begun as soon as the primary leaves were fully expanded; in others the treatments were not started until the plants had produced several compound leaves. At the beginning of the various experiments, dissections were made of representative plants to make certain that none had yet initiated flower primordia. Control plants grown continuously on long photoperiod were included in each experiment.

The effectiveness of each photoperiodic treatment was judged by the number of plants per lot that initiated flower primordia and by the number and location of those so formed. In several of the experiments these observations were made in the terminal bud of the main axis; in others this bud was removed and the observations were made in the terminal buds of branches developed from certain axillary buds. Thus in both types of experiments observations were made in

[1] Morphologist; [2] Associate Physiologist; U.S. Horticultural Station, Beltsville, Maryland.

terminal buds; in one case they were terminals of the main axis and in the other they were terminals of side branches. In both cases flower primordia were also formed in axillary buds below the terminals. The data obtained from these axillary buds, however, contribute little that is not shown in the terminals. This makes it unnecessary in many studies of floral initiation to obtain data from both kinds of buds.

**Experimental results**

The purpose of the first experiment was to study the relative photoperiodic effectiveness of certain individual leaves, parts of leaves, and combinations of two or more leaves. All leaves or parts of leaves except those required in the particular treatment were removed. The plants, defoliated in a variety of ways, were then subjected to 8-hour photoperiods. One-third of the plants of each group received two short photoperiods, another third received four short photoperiods, and the remaining plants were subjected to six short photoperiods. Photoperiodic treatments of all lots were started simultaneously. When these were completed, the plants were returned to photoperiods of 16 or more hours and continued under these conditions until 3 weeks after the beginning of photoperiodic treatments. All plants were then harvested and dissected.

The plants were selected for uniformity of size from a large population. Experimental treatments were started 24 days after the seed was planted. At that time the plants were expanding their fourth compound leaves, and special care was given to the selection of plants whose fourth leaves were of nearly the same size and stage of development.

The total number of plants per lot that had initiated flower primordia was determined and the number and location of flower buds on these plants were recorded. Counts were also made of the nodes in the main axis of each plant.

The third compound leaf on these plants was found to be more effective than any other in causing initiation of flower primordia (table 1). Floral initiation occurred following two short photoperiods in thirty-four of the forty plants in lots 6, 7, 8, and 14, in which lots part or all of the third leaf was present. In the remaining nine partially defoliated lots, none of which involved the third leaf,

only four plants in ninety initiated flower buds. All plants in the undefoliated control (lot 1) bore flower primordia following two short photoperiods.

When longer induction treatments of 4 and 6 short days were given, all plants having a third compound leaf initiated flower

TABLE 1

EFFECT OF VARIOUS METHODS OF DEFOLIATION ON FLORAL INITIATION IN BILOXI SOYBEANS SUBJECTED TO TWO, FOUR, AND SIX PHOTOPERIODS OF 8 HOURS

| LOT NO. | LEAVES PRESENT ON PLANTS DURING INDUCTION TREATMENTS | TOTAL PLANTS PER LOT OF 10 BEARING FLOWER PRIMORDIA AFTER VARIOUS PERIODS OF INDUCTION | | | TOTAL BUDS BEARING FLOWER PRIMORDIA ON MAIN AXIS PER LOT OF 10 AFTER VARIOUS PERIODS OF INDUCTION | | |
|---|---|---|---|---|---|---|---|
| | | 2 DAYS | 4 DAYS | 6 DAYS | 2 DAYS | 4 DAYS | 6 DAYS |
| 1..... | All | 10 | 10 | 10 | 12 | 36 | 54 |
| 2..... | 2 primaries | 0 | 3 | 9 | 0 | 4 | 15 |
| 3..... | 1 primary | 0 | 3 | 7 | 0 | 3 | 9 |
| 4..... | First compound | 0 | 10 | 10 | 0 | 17 | 27 |
| 5..... | Second compound | 0 | 10 | 10 | 0 | 24 | 34 |
| 6..... | Third compound | 9 | 10 | 10 | 10 | 42 | 56 |
| 7..... | Third compound (center leaflet only) | 7 | 10 | 10 | 8 | 41 | 48 |
| 8..... | Third compound (2 lateral leaflets only) | 9 | 10 | 10 | 15 | 33 | 54 |
| 9..... | Fourth compound | 2 | 10 | 10 | 2 | 29 | 45 |
| 10..... | Fourth compound and 1 primary | 1 | 3 | 8 | 3 | 9 | 41 |
| 11..... | Fourth compound and 2 primaries | 0 | 7 | 10 | 0 | 24 | 34 |
| 12..... | Fourth and first compound | 0 | 10 | 10 | 0 | 29 | 42 |
| 13..... | Fourth and second compound | 1 | 10 | 10 | 1 | 19 | 45 |
| 14..... | Fourth and third compound | 9 | 10 | 10 | 11 | 38 | 53 |

primordia. Other lots showed progressive increase with these longer induction treatments, and in the 6-day treatment all plants in most lots and nearly all in the remaining ones formed flower primordia.

Although most plants receiving 6-day induction treatments initiated flower primordia, there was considerable difference between lots as to the number formed. Those in which the third compound leaf was present—either alone, in part, or in combination with other leaves—were equal or superior to all others in this respect.

It is recognized that the experimental treatments involved rather drastic defoliations which may have interfered with differentiation of new structures. Since flower primordia are formed from newly differentiated structures, it is obvious that treatments that stop or retard differentiation may interfere with floral initiation; therefore this possibility must be considered in interpreting the data. A measure of the differences in differentiation of new structures induced on defoliated plants was obtained by determining the total nodes per plant.

Since total nodes per plant in eight of the defoliated lots differed only slightly from the number of those of the undefoliated lot, the data are not presented in detail. The differences among these eight lots were not significant, even at odds as low as 19:1. In five lots, however, there was an appreciable reduction in nodes. These lots were those involving primary leaves, fourth compound leaves, or combinations of these leaves only. The differences in total nodes between these lots and the undefoliated control were in no case greater than two nodes, but they were statistically significant at odds of 99:1.

Analysis of the data on total nodes per plant and total flower primordia initiated per plant seems to show that the differences in formation of flower primordia are caused chiefly by differences in photoperiodic effectiveness of the leaves concerned. Plants having only a fourth compound leaf initiated 4.5 flower buds per plant following a 6-day induction treatment, while those with only a first compound leaf initiated 2.7 flower buds per plant when similarly treated. Removal of all but the fourth compound leaf appreciably reduced the number of nodes per plant, but removal of all but the first compound leaf had little effect. The reduction below the control in the former case was 1.2 nodes and in the latter 0.3 nodes per plant. Although neither of these differences is large, the first is statistically significant at odds of 99:1. In the case of these two lots the plants that formed fewer new structures actually produced a greater number of flower buds than did those of the other lot. The fourth leaf apparently produced a stronger flower-forming stimulus than did the first.

In a second experiment the plants were just expanding the first

compound leaf at the time photoperiodic treatments were begun. As in the former experiment, photoperiodic treatments were applied for 2, 4, and 6 short days. Methods of defoliation are indicated in table 2. Cotyledons were still present on these plants and were not removed.

Where half leaves were left on the plants the cut was made parallel to the midrib, leaving about 1 mm. of leaf blade along one side of the

TABLE 2

EFFECT OF VARIOUS METHODS OF DEFOLIATION ON FLORAL INITIATION IN BILOXI SOYBEANS SUBJECTED TO TWO, FOUR, AND SIX PHOTOPERIODS OF 8 HOURS

| LOT NO. | LEAVES PRESENT ON PLANT DURING INDUCTION TREATMENT | TOTAL NODES PER PLANT AFTER VARIOUS PERIODS OF INDUCTION | | | TOTAL FLOWER-BEARING PLANTS PER LOT OF 10 AFTER VARIOUS PERIODS OF INDUCTION | | | TOTAL BUDS ON MAIN AXIS BEARING FLOWER PRIMORDIA PER LOT OF 10 AFTER VARIOUS PERIODS OF INDUCTION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 DAYS | 4 DAYS | 6 DAYS | 2 DAYS | 4 DAYS | 6 DAYS | 2 DAYS | 4 DAYS | 6 DAYS |
| 1.... | All leaves | 19.0 | 19.4 | 19.4 | 1 | 10 | 10 | 2 | 37 | 49 |
| 2.... | Two primary leaves | 19.1 | 19.0 | 19.1 | 0 | 9 | 10 | 0 | 10 | 29 |
| 3.... | One primary leaf | 18.7 | 18.9 | 18.9 | 0 | 6 | 10 | 0 | 6 | 25 |
| 4.... | Half primary leaf | 17.9 | 18.5 | 18.8 | 0 | 3 | 10 | 0 | 5 | 19 |
| 5.... | One-quarter primary leaf | 17.4 | 18.1 | 18.0 | 0 | 0 | 8 | 0 | 0 | 8 |
| 6.... | First compound leaf | 17.4 | 18.0 | 19.0 | 0 | 10 | 10 | 0 | 21 | 54 |
| 7.... | No leaves | 16.6 | 16.3 | 17.6 | 0 | 0 | 0 | 0 | 0 | 0 |

midrib. Quarter leaves were made by cutting half leaves transversely about midway of the midrib.

Only one plant in all the lots initiated flowers in response to two short photoperiods. Following four short photoperiods, however, all undefoliated plants and all those having only the expanding first compound leaf initiated flower buds. With six short photoperiods nearly all plants formed flower primordia except those in which all leaves were removed.

In this experiment the two primary leaves were approximately half as effective as the expanding first compound leaf in causing floral initiation (table 2). With only the two primary leaves present, twenty-nine flower buds were formed on ten plants in response to a

6-day induction treatment. With only the first compound leaf present, fifty-four flower buds were formed in a lot of ten plants as a result of similar treatment. Plants in these two lots differentiated about the same number of total nodes, but since one lot initiated about half as many flower buds as the other, the data suggest that the leaves of this lot supplied less flower-forming stimulus than those of the other. When the area of the primary leaves was reduced, as in lots 3, 4, 5, and 7, each decrease in area resulted in a decrease in the total number of flower primordia formed per lot and the number of nodes formed per plant. The data seem to indicate that the decrease in flower bud formation resulted from a decreased flower-forming stimulus, rather than from the reduction in the total nodes formed.

Plants of this experiment having only first compound leaves initiated about the same number of flower primordia in response to 6-day induction as plants having all of their leaves (table 2). Apparently the flower-forming stimulus supplied by this one leaf was sufficient to cause the formation of flower primordia in all new buds formed by the plant, and the additional stimulus supplied by the primary leaves when they also were present caused no further increase.

In a third experiment the plants were expanding their third compound leaves when photoperiodic treatments were started and were thus intermediate in size between those of the other two experiments. The second compound leaf of these plants was in about the same stage of maturity as the third compound leaf of experiment 1 and was slightly further advanced than the first compound leaf of experiment 2. The data (table 3) show that when the second compound leaf alone was present on the plant, as many flower buds were formed per lot as when all the leaves were present. When the two lateral leaflets of this leaf were removed, the terminal leaflet alone was still more effective in causing floral initiation than any other leaf on the plants. As a result of a 6-day induction treatment, plants with only their primary leaves initiated thirty-one flower buds per lot; plants with only the first compound leaf initiated fifty-one flower buds per lot; plants with only the second compound leaf initiated sixty-nine flower buds per lot; and those with only the third compound leaf initiated forty-five flower buds per lot. Thus the ability of individual leaves to produce a flower-forming stimulus

increased until the leaf was fully expanded and then decreased. A similar trend was evident in the data of experiment 1.

Although the preceding experiments suggest that the leaves of Biloxi soybeans decrease in their capacity to cause floral initiation as they increase in age, it must be borne in mind that the older the leaf the farther its distance from the tip of the stem. Since the dis-

TABLE 3

EFFECT OF VARIOUS METHODS OF DEFOLIATION ON FLORAL INITIATION IN BILOXI SOYBEANS SUBJECT TO TWO, FOUR, AND SIX PHOTOPERIODS OF 8 HOURS

| Lot no. | Leaves left on plants | Total nodes per plant after various periods of induction | | | Total flower-bearing plants per lot of 10 after various periods of induction | | | Total flower buds on main axis per lot of 10 after various periods of induction | | | Location of lowermost buds containing flower primordia after various periods of induction | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 days | 4 days | 6 days | 2 days | 4 days | 6 days | 2 days | 4 days | 6 days | 2 days | 4 days | 6 days |
| 1 | All | 21.0 | 21.2 | 21.7 | 10 | 10 | 10 | 27 | 51 | 69 | 10.9 | 10.7 | 11.0 |
| 2 | Both primaries | 19.1 | 18.8 | 20.1 | 0 | 10 | 10 | 0 | 13 | 31 | .... | 12.0 | 12.0 |
| 3 | One primary | 18.4 | 18.8 | 19.1 | 0 | 10 | 10 | 0 | 12 | 31 | .... | 11.8 | 11.8 |
| 4 | First compound | 20.7 | 21.2 | 20.9 | 0 | 10 | 10 | 0 | 38 | 51 | .... | 11.5 | 11.4 |
| 5 | Terminal leaflet of first compound | 19.4 | 19.8 | 20.3 | 0 | 10 | 10 | 0 | 26 | 45 | .... | 11.3 | 11.3 |
| 6 | Second compound | 21.1 | 21.2 | 22.0 | 10 | 10 | 10 | 25 | 51 | 69 | 11.0 | 10.8 | 10.6 |
| 7 | Terminal leaflet of second compound | 19.9 | 20.6 | 21.1 | 10 | 10 | 10 | 22 | 42 | 60 | 11.0 | 10.8 | 10.7 |
| 8 | Third compound | 19.1 | 19.2 | 18.6 | 5 | 10 | 10 | 6 | 36 | 45 | 10.0 | 9.7 | 9.9 |

sections in these experiments were confined to the terminal bud, it is possible that the differences in number of flower primordia formed may have been caused by the increased distance through which the stimulus passed, rather than by a decreased capacity of older leaves to stimulate the production of flower buds. Two experiments were designed to determine the relative importance of these two factors.

The plants used in the first of these studies (experiment 4) were expanding the fourth compound leaf when treatments were started. All plants were decapitated by cutting through internode 5 above

the third compound leaf and were then further defoliated and disbudded in four different ways. In half the plants all buds were removed except the one in the axil of the first compound leaf; in the remaining plants all buds were removed except the one in the axil of the third compound leaf. In half the plants of each of these two lots all leaves except the first compound were removed; in the other half all but the third compound were removed. There were thus four different arrangements of the bud and leaf left on the plant. In one case leaf and bud were both located at node 5; in another both were at node 3; in the third the leaf was at node 5 and the bud at node 3; in the fourth the bud was at node 5 and the leaf at node 3. Twenty plants of each of the four groups were then held at 16-hour photoperiods and twenty more were subjected to 8-hour photoperiods. Ten of these received 4 days of treatment while the remaining ten received 6 days. Since none of the plants receiving 16-hour photoperiods initiated any flower primordia, dissection data from them are not presented.

At the time the experiment was started dissections were made of the buds in the axils of the first and third compound leaves of twenty plants similar to those used for experimental treatment. When the experimental lots were dissected 3 weeks after the beginning of treatment, the total nodes differentiated in the branches developed from these buds were, respectively, 14.8 and 15.2. This represented a gain of 9.7 nodes by the former and 9.5 by the latter. The difference between the two is not significant. Both branches bore flower primordia at an average of 3.3 nodes; thus the branches developed from the buds at nodes 3 and 5 made approximately the same amount of growth and their capacities to form flower primordia were similar.

The data of this experiment (table 4) show that the leaf at node 5 caused the formation of more flower primordia than the leaf at node 3. This difference was present in lots receiving either 4- or 6-day treatments and was uninfluenced by the location of the branch on which the observations were made. In the case of lots 1 and 3, it is evident that the age of the leaf and not its distance from the point of flower bud formation was the cause of the difference in number of flower buds formed. In these lots the more effective leaf was

situated two internodes above the point of attachment of the branch on which flower buds were formed, while the less effective leaf was located at the same level as the point of attachment of this branch.

In the next experiment, designed to differentiate between the effects of age of leaf and relative distance from the growing point, the plants were beginning to expand their fifth compound leaves at the beginning of the experiment and were decapitated at the internode below this leaf. In half the plants all leaves were removed

TABLE 4

EFFECT OF VARIOUS METHODS OF DEFOLIATION AND DISBUDDING ON FLORAL INITIATION IN BILOXI SOYBEANS SUBJECTED TO FOUR AND SIX PHOTOPERIODS OF 8 HOURS

| Lot no. | Location of leaf remaining on plant (node) | Location of bud remaining on plant (node) | Total nodes per plant after various periods of induction | | Total flower-bearing plants per lot of 10 after various periods of induction | | Total flower buds per lot on main axis after various periods of induction | |
|---|---|---|---|---|---|---|---|---|
| | | | 4 days | 6 days | 4 days | 6 days | 4 days | 6 days |
| 1..... | 3 | 3 | 14.0 | 15.1 | 9 | 10 | 12 | 40 |
| 2..... | 3 | 5 | 15.3 | 15.5 | 9 | 10 | 10 | 36 |
| 3..... | 5 | 3 | 14.9 | 15.1 | 10 | 10 | 24 | 54 |
| 4..... | 5 | 5 | 14.9 | 15.3 | 10 | 10 | 31 | 50 |

except the fourth compound; in the remainder all leaves but the third compound were removed. All buds were taken off the plants except the one produced in the axil of the second compound leaf. This bud was situated one internode below the older of the two leaves studied in the experiment and two internodes below the younger leaf. Treatment with 8-hour photoperiods was continued 4 days in some lots and 6 days in others.

All the plants subjected to 8-hour photoperiods initiated flower primordia, but those with the younger leaves initiated more than those with the older leaves. Since the flower-forming stimulus from the younger leaf traveled approximately twice as far to the point of flower bud formation as did that from the older leaf, it seems evident that the difference in flower-inducing capacities of the two leaves

was related directly to the ages of the leaves and not to their position with respect to the growing points where flower primordia were formed.

In all these experiments the positions as well as the number of flower primordia were recorded. Differences in the location of the first formed primordia were observed among the various lots of certain experiments. These differences also seemed to be correlated with the age of the leaf, and indicated that young leaves were photoperiodically more effective than old ones. In experiment 3 most plants having only the third compound leaf initiated their first flower primordia at node 10. Most of the undefoliated control plants initiated first flower buds at node 11. Other plants having only the primary leaves formed first flower buds at node 12. In one treatment flower bud formation occurred earlier in the development of the plant than it did in the controls; in the other treatment it occurred later.

These differences in location of first flower primordia seem to be caused by the interaction of two factors, the intensity of the flower-forming stimulus produced by the leaves and the rate of development of new structures from which flower primordia could be formed. When only the third compound leaf was used the formation of new nodes was very much curtailed, but the flower-forming stimulus was of sufficient intensity so that the first flowers were formed at node 10, a point on the stem lower than in any of the other treatments. When only the primary leaves remained on the plant new nodes continued to be formed at about the same rate as in the previous case, but the flower-forming stimulus was apparently less intense because the buds at nodes 10 and 11 produced only vegetative structures and the first flower primordia were formed at node 12.

In the controls the first flower primordia were formed at node 11. In this case there was no curtailment of growth because no leaves were removed. Although more flower buds were initiated by these plants than by those having only the third compound leaf, the first flower buds were formed at higher positions on the plant in the former than in the latter. The difference in level was only one node but it was very consistent among all the plants. Differences in the same direction have been found between lots similarly treated in

several other experiments. The reason for this apparently earlier formation of flower primordia on a defoliated than on an undefoliated plant is not yet clear. It seems probable, however, that at the time treatments were started the structures at node 10 had about reached a point in their development beyond which they could no longer respond to flower-forming stimulation. In the case of the controls the primordia at node 10 apparently developed beyond this point before the flower-forming stimulus could reach them and cause formation of flower primordia. In the plants defoliated to a third compound leaf, however, the development of these structures seems to have been retarded or temporarily stopped, permitting the flower-forming stimulus to reach node 10 in time to cause floral initiation when growth at that point was resumed. Whether or not this explanation is correct must be determined by further study.

## Discussion

The data of these experiments suggest that every expanded leaf on a Biloxi soybean plant subjected to photoperiodic stimulation is able to cause the formation of flowers at the growing points. Apparently the various leaves differ considerably in their flower-inducing capacities; but if all are present and subjected to short photoperiods of natural light, the flower-forming stimulus is adequate to result in flower formation in all the new buds formed.

The youngest fully expanded leaf is the most effective one in causing flower bud formation. In experiments 1, 2, and 3 compound leaves 3, 1, and 2 respectively were in this stage of development and caused the initiation of more flower primordia than any other leaf on the plant. Even when they were present alone they induced the formation of as many flower buds as when all the leaves were present. This indicates that they were able to supply the plant with sufficient stimulation to cause floral initiation in all newly formed buds, without the help of the other leaves.

The very young leaves that are just beginning to expand increase very rapidly in their capacity to cause floral initiation—until they are fully expanded. After they pass this stage they apparently supply progressively less flower-forming stimulus.

**Summary**

1. Individual leaves on a Biloxi soybean when subjected to photoperiodic stimulation differ in their capacities to cause floral initiation.

2. The most effective leaf on the plant is the one that has most recently attained its full size. Young leaves increase in their capacity to effect floral initiation until they attain full size, after which they gradually decline in effectiveness.

3. The most active leaf, operating alone, is able to cause initiation of as many flower buds per plant as are formed when all leaves function simultaneously.

4. The capacities of different leaves to supply a flower-forming stimulus is correlated with their relative states of maturity and not with their distances from the growing points where flowers are formed. When the flower-forming stimulus from the third compound leaf passes downward through two internodes to the bud in the axil of the first compound leaf, it induces the formation of more flower primordia there than does the stimulus from the first compound leaf which is situated immediately adjacent to the bud.

5. The flower-forming stimulus produced by the leaves of soybeans moves readily both up and down the stem.

U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. BOT. GAZ. 100:374–387. 1938.
2. CAJLACHJAN, M. H., On the mechanism of photoperiodic reaction. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 1:89–93. 1936.
3. HAMNER, K. C., and BONNER, J., Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1938.
4. KNOTT, J. E., Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. 31:152–154. 1934.
5. LUBIMENKO, V. N., and BOUSLOVA, E. D., Contribution à la theorie du photoperiodisme. II. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. 14: 149–152. 1937.

# GROWTH AND DEVELOPMENT OF THE EMBRYO AND FRUIT OF THE PEACH AS AFFECTED BY RINGING AND DEFOLIATION OF THE BRANCHES[1]

H. B. TUKEY AND F. A. LEE

(WITH FOUR FIGURES)

## Introduction

Peach fruits develop in three stages (13), as shown in figure 1. In stage I the pericarp increases rapidly in size for a definite period following full bloom (about 49 days). The stage is of relatively uniform duration for all varieties, regardless of season of fruit ripening. In stage II, development is retarded for a variable period, depending upon the variety. For a variety which ripens the fruit early, as Greensboro, stage II is of only 5 days' duration; for a mid-season variety, as Elberta, it is 28 days; and for a late-ripening variety, as Chili, it is 42 days. In stage III, known as the final swell, the pericarp increases rapidly in size to fruit ripening. The difference between an early-ripening and a late-ripening variety, therefore, is largely a matter of duration of stage II.

Development of the embryo does not parallel that of the pericarp. During stage I the embryo remains microscopic. At the beginning of stage II the embryo begins a period of rapid enlargement, which continues until it reaches maximum size for the variety. In the case of a very early-ripening variety, stage III is initiated before the embryo has completed the period of rapid development. The embryo fails to fill the integuments, the nucellus and integuments collapse, and a shriveled or abortive seed results. In the case of a late-ripening variety, stage III does not begin until the embryo is nearly the size of the integuments. Such seeds are viable and will germinate following a period of after-ripening.

In an earlier publication (12) it was shown that varieties of sweet cherry (*Prunus avium* L.) and of peach (*P. persica* Batsch.) which ripen the fruit early tend to have abortive embryos. Further, the

[1] Journal article no. 361 of the New York State Agricultural Experiment Station.

earlier the season of fruit ripening the earlier the stage in development at which the embryos are aborted. The question was at that time raised whether early-ripening might induce embryo abortion, or whether abortion might induce early ripening. In a subsequent paper (14) it was shown that destruction of the embryo in stage III hastened ripening of the fruit, thus establishing the point that abortion of the embryo induces earlier ripening, and showing a direct relationship between a developing fruit and a developing embryo within the fruit. A question immediately suggested by these facts is what effect a check in development of the fruit would have in turn upon the development of the embryo within the fruit. The results presented in this paper attempt to answer this question and carry with them certain suggestions as to the part a fruit may play in embryo development.

## Material and methods

The Elberta and Ward Late varieties of peach were used, the former ripening in late mid-season (128 days after full bloom) and the other late (142 days after full bloom). The studies were conducted during the growing seasons of 1936 and 1937 in the varietal orchards of the New York State Agricultural Experiment Station and in the commercial orchard of Willis Henderson at Dresden, New York. Both were good crop years, providing what would be considered good growing conditions and typical growth for the varieties.

The growth curves of the varieties have been established during several seasons at Geneva, New York. The fact that the blooming period is short (36–48 hours), so that fertilization occurs and fruits begin development at approximately the same time, makes it possible to secure a large number of fruits for sampling which are nearly identical in development at any one time.

Since rapid enlargement and rapid mobilization of storage materials in the embryo do not occur until the fruit has entered stage II, the experimental work was not begun until then. Branches of good vigor, $\frac{3}{8}$–$1\frac{1}{2}$ inches in diameter, and carrying similar numbers of fruits per unit size of branch, were selected. In both seasons the distribution of fruit on the trees was fairly uniform.

Ringing was done with a sharp knife, a ring of bark $\frac{1}{2}$-inch wide

being removed, and the exposed wood thoroughly scraped to remove any meristematic tissue which might tend to bridge the gap. Both grafting wax and adhesive grafting tape were used to cover the wounds, both proving equally effective; the latter being more easily applied. All branches in the experiment were inclosed in large cheesecloth bags both to protect the fruit and to catch any which might drop. The fruits per branch varied in number from 11 to 63, with a mean of 27.

Five methods of treatment were used: (1) All current season's wood growth was removed, branch was ringed 2 feet from the end, and all leaves removed from section distal to the ring. (2) Similar to treatment 1 but no leaves removed. (3) No current season's wood growth removed, branch ringed at both 2 and 4 feet from the end, and all leaves removed from the section between the rings. (4) Similar to treatment 3 but no leaves removed. (5) Neither ringed nor defoliated. This combination of treatments made it possible to compare the possible effects from hormones or growth substances manufactured in the growing points of the twigs and the interruption in the movement of materials past the rings. In some instances it was possible to arrange for treatments to be on the same branch, that is, treatments 1, 3, and 5; 1, 4, and 5; 2, 3, and 5; and 2, 4, and 5.

Five sets of experiments were run as follows, the termination of each being determined by the onset of fruit abscission from ringed

| YEAR, PERIOD, AND VARIETY | DURATION OF TEST | |
|---|---|---|
| 1936 A, Ward Late | Beginning of stage II (July 1) | to middle of stage II (July 15) |
| B, Ward Late | Middle of stage II (July 15) | to end of stage II (August 8) |
| 1937 A, Elberta | Beginning of stage II (July 9) | to middle of stage II (July 24) |
| B, Elberta | Middle of stage II (July 24) | to middle of stage III (August 24) |
| C, Elberta | Middle of stage III (August 24) | to end of stage III (September 18) |

and defoliated branches. As will be noted, a new experiment was begun at the time each preceding experiment ended, thus giving a continuous record from the end of stage I through stages II and III

to fruit ripening. The beginning of each experiment with the Elberta variety in 1937 is indicated in figure 1.

Samples of fruit and twigs were collected at both the beginning and end of each period of ringing and defoliation. Fruits, seeds, and embryos were measured and chemical analyses made of twigs, fleshy pericarp, stony pericarp, entire seeds, and embryos. Analyses were also attempted separately of integuments, nucellus, and endosperm, when sufficient material could be secured. Great difficulty was experienced in separating these tissues in quantity and in doing the

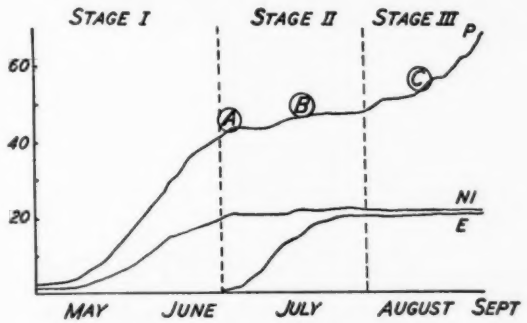



FIG. 1.—Growth of pericarp (*p*), nucellus and integuments (*ni*), and embryo (*e*) of peach from pre-bloom to ripening, showing the three characteristic stages of fruit development and periods at which ringing-and-defoliation (*A*, *B*, *C*) was done.

work with sufficient rapidity to preclude loss of moisture and chemical change.

Determinations were made of total solids, reducing sugars as glucose, sucrose, ether extract (fat), and total nitrogen. In preparing the material for chemical analysis, the seeds were removed from the fruits, and the integuments, remaining nucellus, and endosperm dissected away, leaving the embryos. These were immediately weighed and divided into three duplicate samples for determination of fat, nitrogen, and sugars. The samples for ether extract (fat) were dried at 95° C. for 2 hours and then placed in a vacuum oven at 70° C. until they reached constant weight. The percentage of moisture was determined from these samples. The samples for sugar were placed directly in 80 per cent alcohol.

Fat was determined by the common ether extraction method; sugars by the method involving extraction by 80 per cent alcohol; and sucrose after inversion with invertase. The Munson and Walker method by direct weighing was used for all sugars. The determinations for reducing sugars were calculated as glucose. For nitrogen the Gunning method was used. Calculations were made on the fresh weight, dry weight, and the unit basis.

The results for both seasons and with both varieties are similar. Accordingly complete data are given for the Elberta variety during the season of 1937, as representative, and any variations which occurred with the Ward Late variety and in 1936 are discussed in the text.

## Results

### Morphological changes

The morphological changes which occurred with the Ward Late variety in 1936 and the Elberta variety in 1937 were similar. There seemed to be no differences in development of fruit or seed, whether on terminal sections of ringed branches or on sections between two rings, whether on ringed or unringed non-defoliated branches, or whether on branches involving several treatments at once (as outlined under Methods). Any hormone or growth substance which may have been manufactured in the leaves or terminal growing points—and which may play a part in fruit and seed development—was either able to pass the rings or was otherwise not affected by the treatments. On the basis of the responses it was thus possible to place the treatments in two groups, (*a*) ringed-and-defoliated and (*b*) check.

The results are shown in table 1. Several points should be emphasized. Ringing and defoliation either checked or greatly retarded the increase in size of the fleshy pericarp, although bringing about earlier coloring and earlier ripening. They retarded the differentiation of the cell walls of the stony pericarp, so that they failed to harden to the same degree as in the check. They brought about earlier differentiation of the integuments, as shown by browning. They failed to have appreciable effect upon the rate of increase and ultimate size of the embryo, as measured in length, up to the time

TABLE 1

EFFECT OF RINGING AND DEFOLIATION UPON DEVELOPMENT OF ELBERTA PEACH EMBRYO (1937)

| DATE | LENGTH OF FLESHY PERICARP (MM.) | CONDITION OF STONY PERICARP | LENGTH OF NUCELLUS AND INTEGUMENTS (MM.) | LENGTH OF EMBRYO (MM.) | DEVELOPMENT (STAGE) |
|---|---|---|---|---|---|
| | PERIOD A | | | | |
| July 9 (at time of ringing and defoliation) | 39.0 | Hardening | 20.0 | 2.5 | Beginning of II |
| July 24 | | | | | |
| On ringed-and-defoliated branches | 42.0 | Less hard than check | 20.0 | 16.0 | Middle of II |
| On check branches | 42.0 | Hard | 20.0 | 16.0 | Middle of II |
| | PERIOD B | | | | |
| July 24 (at time of ringing and defoliation) | 42.0 | Hard | 20.0 | 16.0 | Middle of II |
| August 24 | | | | | |
| On ringed-and-defoliated branches | 42.0 (yellow) | Hard | 20.0 (integuments brown) | 19.5 | Middle of III |
| On check branches | 45.0 (green) | Less hard than check | 20.0 (integuments brown) | 19.5 | Middle of III |
| | PERIOD C | | | | |
| August 24 (at time of ringing and defoliation) | 45.0 | Hard | 20.0 | 19.5 | Middle of III |
| September 18 | | | | | |
| On ringed-and-defoliated branches | 47.0 (highly colored, lacking in flavor) | Hard | 20.0 | 19.5 | End of III |
| On check branches | 54.0 | Hard | 20.0 | 19.5 | End of III |

the fruit abscissed. But the embryos from ringed-and-defoliated branches seemed less firm than those from check branches, and the weight per unit was less, as shown in table 2 and as discussed later.

TABLE 2

UNIT WEIGHTS OF TISSUES OF FRUIT AND SEED OF ELBERTA PEACH ON RINGED-AND-DEFOLIATED BRANCHES; FRESH WEIGHT BASIS (MG.)

| TREATMENT | PERIOD A | | PERIOD B | | PERIOD C | |
|---|---|---|---|---|---|---|
| | JULY 7 | JULY 24 | JULY 24 | AUG. 24 | AUG. 24 | SEPT. 18 |
| | FLESHY PERICARP | | | | | |
| Check | 14,632 | 18,897 | 18,897 | 34,736 | 34,736 | 61,306 |
| R and D* | 14,632 | 15,901 | 18,897 | 24,314 | 34,736 | 40,589 |
| | STONY PERICARP | | | | | |
| Check | 4748 | 5690 | 5690 | 6108 | 6108 | 5340 |
| R and D | 4748 | 4847 | 5690 | 5208 | 6108 | 5396 |
| | ENTIRE SEED | | | | | |
| Check | 638 | 668 | 668 | 690 | 690 | 685 |
| R and D | 638 | 653 | 668 | 646 | 690 | 651 |
| | EMBRYO | | | | | |
| Check | <0.1 | 349 | 349 | 567 | 567 | 595 |
| R and D | <0.1 | 340 | 349 | 571 | 567 | 591 |

* Ringed and defoliated.

SHUHART (10) reports somewhat similar results with the pecan, in which defoliation failed to stop the enlargement of the embryo and the filling of the nut.

## CHANGES IN COMPOSITION

The changes in size and differentiation already discussed are reflected in the changes in weight per unit, in total solids, and in

chemical composition. In table 2 are shown the mean fresh weights per unit, secured by averaging the many hundreds of units taken for chemical analyses. Table 3 gives the mean milligrams per unit of total solids, reducing substances as glucose, sucrose, ether extract, and protein (nitrogen $\times 6.25$), secured by dividing the total amount of a constituent determined in a sample by the number of units contained in it. Percentages on both the fresh weight and dry weight basis have been computed, but since they add nothing significant to the data and since they may be computed from the figures given, they are omitted from this paper.

Although endosperm, nucellus, and integuments were separated from one another in so far as possible and then analyzed, the data are omitted because of the difficulties of sampling as mentioned in the discussion of material and methods. It is possible, however, by subtracting the analyses of the embryo from the analyses of the total seed (table 3), to secure an approximation of the changes which occur in the tissues of endosperm, nucellus, and integument, taken together.

### Changes in fleshy pericarp, stony pericarp, and embryo

Fresh weight per unit.—The points of particular interest with reference to the fresh weight per unit are (*a*) the retarded increase in fresh weight of the fleshy pericarp on ringed-and-defoliated branches, (*b*) the retarded increase and more rapid loss in fresh weight of the stony pericarp, and in sharp contrast (*c*) the nearly identical increase in fresh weight of the embryo on both ringed-and-defoliated and check branches (table 2 and fig. 2).

Total solids per unit.—The changes in total solids, or dry weight, shown in table 3 and figure 2 are in many ways the most significant figures in this paper. In the case of the fleshy pericarp, whatever increase in fresh weight did occur on ringed-and-defoliated branches was due entirely to increase in moisture. Actually the fleshy pericarp lost in total solids on ringed-and-defoliated branches as contrasted with the large gain on check branches, at the same time that the total fresh weight per unit was increasing.

In the case of the stony pericarp, the changes are somewhat different from those in the fleshy pericarp and easily may be misinter-

TABLE 3

CHEMICAL ANALYSIS OF TWIGS AND TISSUES OF FRUIT AND SEED OF ELBERTA PEACH ON RINGED-AND-DEFOLIATED BRANCHES IN COMPARISON WITH THE CHECK; UNIT WEIGHT BASIS OF FRESH MATERIAL (MG.)

| PERIOD AND DATE | TOTAL SOLIDS | | REDUCING SUBSTANCE AS GLUCOSE | | SUCROSE | | ETHER EXTRACT | | PROTEIN (N×6.25) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CHECK | R AND D* | CHECK | R AND D | CHECK | R AND D | CHECK | R AND D | CHECK | R AND D |
| | TWIGS† | | | | | | | | | |
| A 7/9 | 5263 | 5263 | 139 | 139 | 15 | 15 | 190 | 190 | 206 | 206 |
| A 7/24 | 5722 | 5252 | 157 | 105 | 38 | 23 | 192 | 142 | 200 | 215 |
| B 7/24 | 5722 | 5722 | 157 | 157 | 38 | 38 | 192 | 192 | 200 | 200 |
| B 8/24 | 5465 | 4986 | 142 | 75 | 34 | 8 | 148 | 127 | 175 | 163 |
| C 8/24 | 5465 | 5465 | 142 | 142 | 34 | 34 | 148 | 148 | 175 | 175 |
| C 9/18 | 6015 | 5780 | 132 | 140 | 72 | 15 | 140 | 146 | 206 | 69 |
| | FLESHY PERICARP | | | | | | | | | |
| A 7/9 | 2411 | 2411 | 559 | 559 | 196 | 196 | 70.2 | 70.2 | 191 | 191 |
| A 7/24 | 2834 | 2083 | 667 | 514 | 306 | 212 | 110.0 | 87.5 | 303 | 248 |
| B 7/24 | 2834 | 2834 | 667 | 667 | 306 | 306 | 110.0 | 110.0 | 303 | 303 |
| B 8/24 | 4411 | 2266 | 1056 | 343 | 848 | 168 | 76.4 | 43.8 | 349 | 288 |
| C 8/24 | 4411 | 4411 | 1056 | 1056 | 848 | 848 | 76.4 | 76.4 | 349 | 349 |
| C 9/18 | 7418 | 3775 | 2048 | 958 | 2520 | 898 | 80.3 | 70.2 | 526 | 354 |
| | STONY PERICARP | | | | | | | | | |
| A 7/9 | 1051 | 1051 | 145.0 | 145.0 | 25.6 | 25.6 | 23.9 | 23.9 | 81.2 | 81.2 |
| A 7/24 | 2729 | 1141 | 51.8 | 162.0 | 15.9 | 13.1 | 15.4 | 15.5 | 127.0 | 85.3 |
| B 7/24 | 2729 | 2729 | 51.8 | 51.8 | 15.9 | 15.9 | 15.4 | 15.4 | 127.0 | 127.0 |
| B 8/24 | 4446 | 2720 | 34.9 | 18.3 | 9.8 | 1.6 | 15.3 | 14.1 | 82.5 | 39.1 |
| C 8/24 | 4446 | 4446 | 34.9 | 34.9 | 9.8 | 9.8 | 15.3 | 15.3 | 82.5 | 82.5 |
| C 9/18 | 4066 | 4042 | 15.0 | 10.3 | 5.3 | 3.2 | 9.2 | 9.3 | 29.3 | 31.7 |

* Ringed and defoliated.

† 10 gm. unit for comparative purposes.

TABLE 3—*Continued*

| Period and date | Total solids | | Reducing substance as glucose | | Sucrose | | Ether extract | | Protein (N×6.25) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Check | R and D* | Check | R and D | Check | R and D | Check | R and D | Check | R and D |
| | Entire seed | | | | | | | | | |
| A 7/9 | 45.3 | 45.3 | 2.8 | 2.8 | 0.8 | 0.8 | 1.1 | 1.1 | 7.9 | 7.9 |
| A 7/24 | 64.2 | 65.1 | 4.5 | 3.9 | 2.9 | 2.6 | 3.0 | 3.6 | 27.4 | 27.6 |
| B 7/24 | 64.2 | 64.2 | 4.5 | 4.5 | 2.9 | 2.9 | 3.0 | 3.0 | 27.4 | 27.4 |
| B 8/24 | 255.9 | 177.7 | 7.5 | 2.9 | 9.0 | 8.9 | 87.1 | 38.5 | 92.3 | 77.7 |
| C 8/24 | 255.9 | 255.9 | 7.5 | 7.5 | 9.0 | 9.0 | 87.1 | 87.1 | 92.3 | 92.3 |
| C 9/18 | 382.4 | 325.9 | 7.0 | 5.7 | 13.2 | 14.7 | 180.9 | 125.7 | 98.3 | ..... |
| | Embryo | | | | | | | | | |
| A 7/9 | < 0.1 | < 0.1 | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| A 7/24 | 26.7 | 27.2 | 2.8 | 1.5 | 1.8 | 2.1 | 1.3 | 1.7 | 15.1 | 15.1 |
| B 7/24 | 26.7 | 26.7 | 2.8 | 2.8 | 1.8 | 1.8 | 1.3 | 1.3 | 15.1 | 15.1 |
| B 8/24 | 223.0 | 152.0 | 4.6 | 2.1 | 7.5 | 8.5 | 83.0 | 34.9 | 86.5 | 73.5 |
| C 8/24 | 223.0 | 223.0 | 4.6 | 4.6 | 7.5 | 7.5 | 83.0 | 83.0 | 86.5 | 86.5 |
| C 9/18 | 349.0 | 294.0 | 4.4 | 4.9 | 12.8 | 14.2 | 174.4 | 120.0 | 94.0 | 89.9 |

preted. On check branches during periods A and B the gain in total solids per unit is actually greater than the gain in total fresh weight per unit. While at first this may seem anomalous, it means merely that the stony pericarp in its hardening processes is losing moisture at the same time that it is gaining in total solids, resulting in a net increase in total weight per unit. For example, while the stony pericarp increased 942 mg. in fresh weight during period A (table 2) it gained 1678 mg. in total solids (table 3). Therefore it must have lost moisture equivalent to the difference between these two figures, or 736 mg. Likewise during period B the gain of 1717 mg. in total solids accompanied by a gain in total fresh weight of 418 mg. means an actual loss of 1299 mg. in moisture during the period. Finally, during period C the stony pericarp lost both in moisture and in total solids,

388 mg. of the former and 380 mg. of the latter for a total loss of 768 mg. per unit fresh weight. The figures thus agree with the morpho-

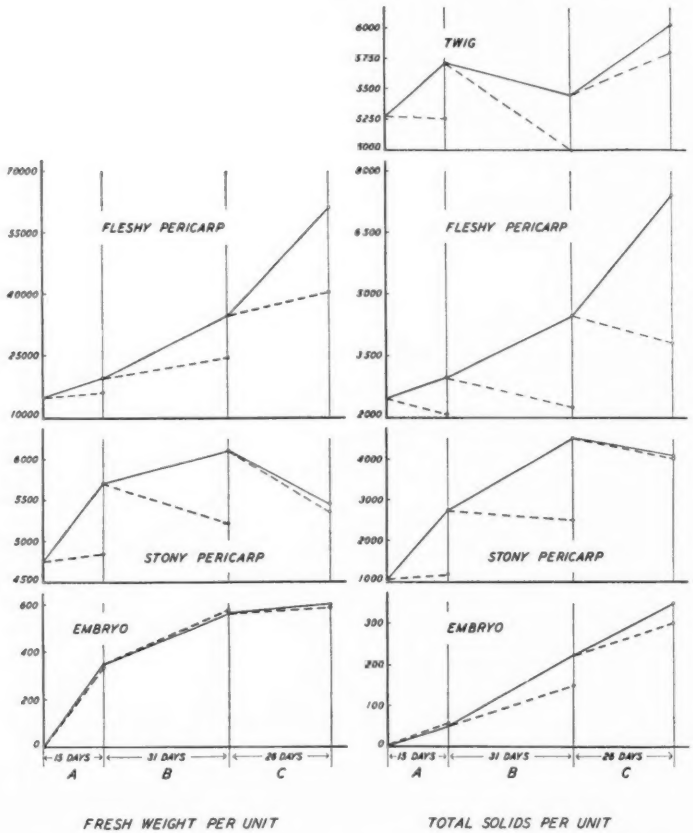



FIG. 2.—Changes per unit (mg.) in fresh weight and in total solids in twigs, fleshy pericarp, stony pericarp, and embryo during three periods of ringing and defoliation (broken line) in comparison with the check (solid line).

logical determinations that the cell walls are greatly thickened and hardened during stage II, and that during stage III there is drying out and increase in brittleness.

The effect on the stony pericarp of ringing and defoliation during

stages II and III was to prevent almost entirely any increase in total solids at a time when such increase was progressing rapidly on check branches. Furthermore, it increased only slightly the loss in total dry weight which occurred in the latter part of stage III on both check and ringed-and-defoliated branches. Here again the figures agree with the morphological findings that the stony pericarp on ringed-and-defoliated branches was checked in differentiation during stage II and did not harden, but was not noticeably affected during the latter part of stage III.

The endosperm, nucellus, and integuments taken together reach the maximum in size and dry weight at the beginning of period A. The moisture content (92.9 per cent) is high, however, the highest of any of the tissues reported here. During subsequent stages on check branches, owing to digestion of the nucellus and later of the endosperm, both the weight per unit and the dry weight steadily declined. Ringing and defoliation increased the loss of solids, particularly during period B, when the embryo was making greatest increase.

The effect of ringing and defoliation upon the embryo is more simply stated. Total solids increased on both ringed-and-defoliated and on check branches, although the accumulation on ringed-and-defoliated branches was not so great as on non-defoliated ones. Whether this was due to a slower rate of accumulation throughout the period, to a retardation toward the end of each period, or to a combination of both, the data give no clue. Since the fruits were left on the tree until they began to absciss, however, one might suspect that the differences were more likely due to a check in accumulation near the end of the period. The figures again agree with the observation that embryos on ringed-and-defoliated branches were more flaccid than those on check branches, even though of equal size and weight.

REDUCING SUBSTANCES AS GLUCOSE PER UNIT FRESH WEIGHT.—Reducing substances (table 3 and fig. 3) increased per unit of fleshy pericarp on check branches during all three periods and decreased on ringed-and-defoliated branches. The greatest loss following ringing and defoliation occurred during period B, when the embryo was making most rapid increase.

In the stony pericarp, reducing substances were found in about the same percentage as in the fleshy pericarp. Instead of increasing in

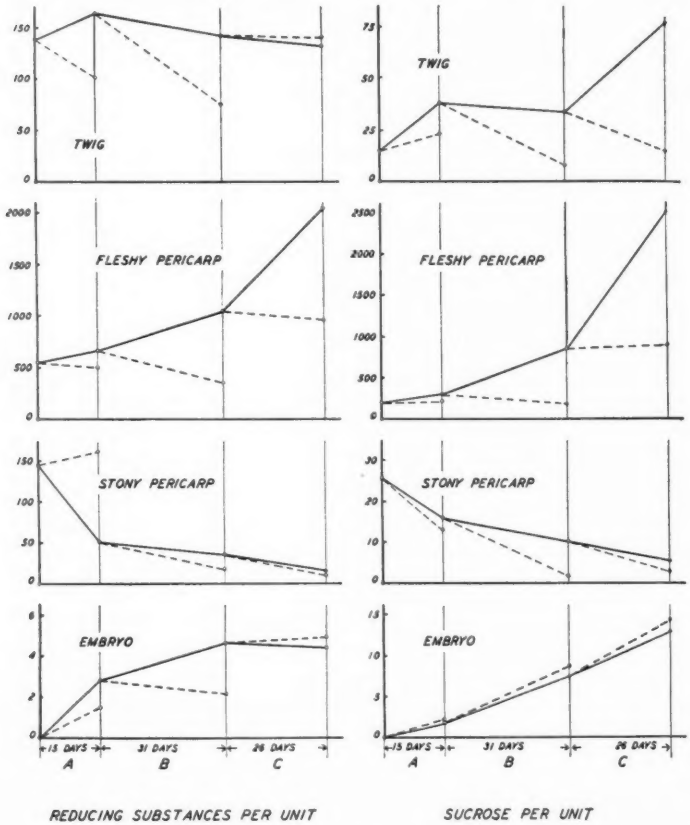



FIG. 3.—Changes per unit (mg.) in reducing substances as glucose and in sucrose in twigs, fleshy pericarp, stony pericarp, and embryo during three periods of ringing and defoliation (broken line) in comparison with the check (solid line).

amount, however, they decreased, both on check and on defoliated branches—except during period A. In that period, both in 1936 and in 1937, the immediate effect of defoliation was to increase the amount of reducing substances in the stony pericarp. No satisfac-

tory explanation of this occurrence or of its significance has been found. The loss of reducing substances during periods B and C on both check and ringed-and-defoliated branches is also of interest.

SUCROSE.—The gains and losses of sucrose (table 3 and fig. 3) closely follow those for reducing substances in both fleshy and stony pericarp on check branches. The total amounts of sucrose per unit, however, were slightly less than those for reducing substances in the fleshy pericarp until just before fruit ripening, and much less in the stony pericarp.

Ringing and defoliation failed to retard the accumulation of sucrose in the fleshy pericarp during the first part of stage II. In fact there was a slight increase, suggestive of the increase in reducing substances in the stony pericarp during the same period following like treatment. Subsequently, either the accumulation of sucrose was retarded or else a loss occurred, at a time when there was rapid accumulation in the fleshy pericarp.

In the stony pericarp on check branches sucrose decreased steadily from the end of stage I to fruit ripening, and the decrease was accelerated by ringing and defoliation. In the endosperm, nucellus, and integuments, sucrose was reduced to traces. In the embryo, in contrast, sucrose accumulated steadily during this period on both check and ringed-and-defoliated branches, the accumulation on the latter being even greater than on check branches. It is of interest to note that the effect of ringing and defoliation during the first part of stage II resulted in an increase in sucrose in the embryo as compared with the check, at the same time that it induced an increase in sucrose in the fleshy pericarp and an increase in glucose in the stony pericarp.

STARCH.—No starch was found in either the embryo, stony pericarp, or ripe fruit, although small amounts were found in the fleshy pericarp in early stages of fruit development when the fruit was still green. It was possible to secure a value for starch by some quantitative chemical methods for starch determination in tissues other than that of the green fleshy pericarp, but since no starch grains could be detected qualitatively, it is inferred that the value obtained is for some material other than starch. Possibly some of the values reported as starch by other workers may also be for some other material (**7, 8**).

ETHER EXTRACT.—The amount per unit of ether extract (table 3 and fig. 4) in both fleshy and stony pericarp on check branches was less than the amount of reducing substances. It was also less than the amount of sucrose in the fleshy pericarp and nearly equal to the amount of sucrose in the stony pericarp. There was slight accumulation in the fleshy pericarp during the first part of stage II, followed by decline and leveling off as fruit ripening was approached. The effect of ringing and defoliation was to retard accumulation and to accelerate loss.

In the stony pericarp the amount of ether extract was very low, and during all periods of the experiments there was a steady loss. The losses on check branches were nearly identical with those on ringed-and-defoliated branches. In the embryo, in sharp contrast, ether extract increased steadily on both check and ringed-and-defoliated branches until fruit ripening. At that time ether extract was the greatest single storage constituent of the embryo, 29.31 per cent on fresh weight basis and 49.95 per cent on dry weight basis. The total accumulation was less on defoliated than on check branches. This may have been due to a slower rate of mobilization on defoliated branches than on check branches, or to an earlier cessation of mobilization during this period, or to a combination of both. Since samples were not gathered until the fruit was ready to drop, it would seem that the second suggestion is plausible. The significant point, however, is that the embryo continued to mobilize and to accumulate so much ether extract following defoliation of the branches.

PROTEIN.—The changes in protein (table 3 and fig. 4) in the fleshy and stony pericarp followed in general the trend for sucrose, although the relative quantities of each in the different tissues varied. Thus on check branches there was a slow but steady gain in protein in the fleshy pericarp and an initial gain in the stony pericarp, followed by losses. The effect of defoliation was to reduce the accumulation of protein in the first part of stage II and to increase the losses in the last of stage II and throughout stage III.

In the embryo the trend was similar to that of ether extract, in which there was an increase in protein even on ringed branches. The greatest increase in protein occurred, however, before the last part

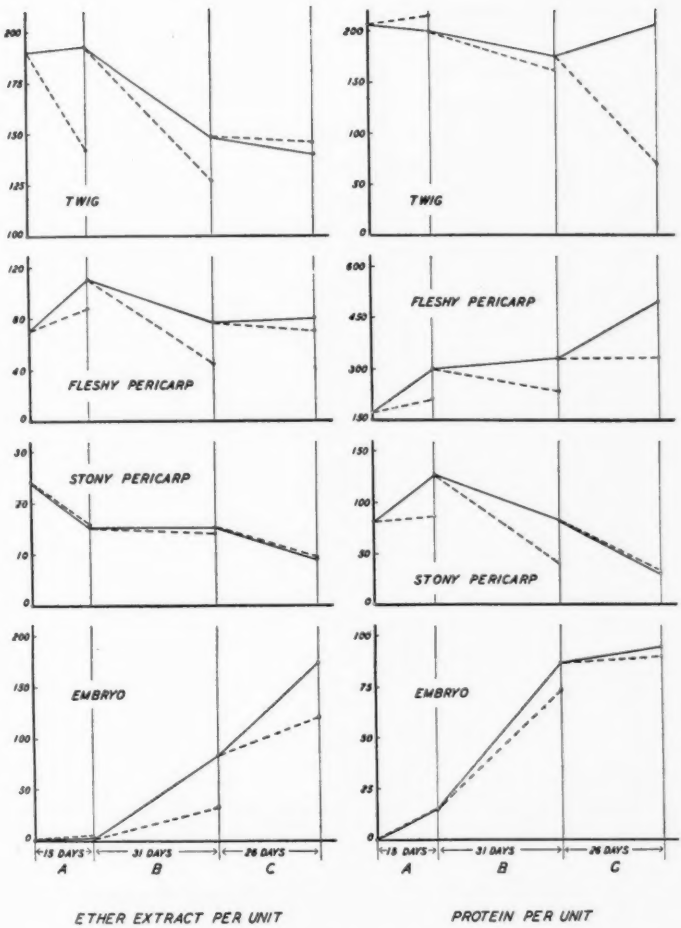



FIG. 4.—Changes per unit (mg.) in ether extract and protein in twigs, fleshy pericarp, stony pericarp, and embryo during three periods of ringing and defoliation (broken line) in comparison with the check (solid line).

of stage III, whereas in the case of ether extract the accumulation continued in about the same amount until the end of stage III. The significant feature is not only that the embryo continued to mobilize protein even after ringing and defoliation, but that the total amounts accumulated on check and on ringed-and-defoliated branches were so similar. Thus during period A the increase on fresh weight basis was from a trace to 4.33 per cent on check branches and to 4.46 on ringed-and-defoliated branches; during period B, 15.26 and 12.88 per cent, respectively; and during period C, 15.80 and 15.20 per cent, respectively.

## Discussion

It has been suggested in the literature that embryos may develop within fruits which have abscissed prematurely, indicating that fruit dropping in such instances is not due to failure of the embryo but to the nutritional condition of the tree (2). BRADBURY (1) concluded that embryos of sour cherries may continue development in the embryo sac of an aborting fruit for some time after the ovary has been checked; and TUKEY (15) has shown the potential viability of abortive embryos of apple, pear, peach, cherry, and plum when placed in artificial culture. The data presented in this paper substantiate these suggestions by showing that a check in development of the fruit does not result in immediate check in development of the embryo.

There are implications from the data that even under normal conditions the fruit may play a physiological role and that the materials of the embryo may be derived either entirely or in part from the fruit, as though the fruit served as a reservoir from which materials were moved into the embryo at the time of rapid development. THOR and SMITH (11) have analyzed such a possibility with the pecan. Considering first the seed alone, they conclude "the idea . . . . that the kernel of the pecan first fills with carbohydrate material which rapidly changes over to oil during the ripening process is entirely without a quantitative basis." This appears true also for the peach, for the total solids in the entire seed just prior to the period of rapid enlargement of the embryo are only 45.3 mg., whereas the total solids in the embryo alone at maturity are 349 mg.,

to say nothing of their higher energy value compared with that of the materials in the endosperm, nucellus, and integuments.

THOR and SMITH have also shown that it is impossible on a quantitative basis to prove other than "that practically all of the oil content of the pecan kernel is formed from materials brought into the fruit from other parts of the tree at the time of oil formation." VALLEE (**16**) concluded that the reducing sugars and sucrose which are accumulated in the pericarp of the almond are translocated to the embryo and stored as fat. In studies with the date, LLOYD (**6**) also considered the possibilities of a nutritive relation between embryo, seed, and carpel. SHUHART (**10**) has done similarly for the pecan, and LOTT (**7, 8**) for the peach.

Computations of the data for the peach presented in this paper show that it is theoretically possible for an appreciable quantity of some of the substances which comprise the embryo to be derived from the fruit. Thus on check branches the losses in dry weight from the fruit during development, in fact from the stony pericarp alone, are more than sufficient to equal the increase in dry weight in the embryo. Likewise the losses of reducing substances and sucrose from the stony pericarp are sufficient to equal the increase in the embryo. On the other hand, the losses of ether extract from both the fleshy and stony pericarp together are not sufficient to equal the increase in ether extract in the embryo; yet if the excesses of reducing substances and sucrose above the amount gained by the embryo are calculated as glycerol trioleate, representing ether extract, it is possible to realize a value which makes it theoretically possible for the ether extract gained by the embryo to have been derived from the fruit.

The complication lies in the probable conversion of one form of material into another within the same tissue of the fruit, as suggested by FINCH and VAN HORN (**5**) and SHUHART (**10**) for the pecan. Thus the cell walls of the stony pericarp of the peach as here reported become greatly thickened and highly lignified during the period when the greatest loss in dry weight occurs. This loss amounts to 8.5 per cent on dry weight basis and could be accounted for by the dehydration accompanying polymerization of simple materials to more complex ones. If the change was from glucose to a disac-

charide, a loss of 5 per cent could be accounted for; if to cellulose, 10 per cent; while if to lignin, over 10 per cent.

In the case of protein, the loss for the entire fruit does not equal the gain by the embryo, so that the nitrogenous material received by the embryo must come from outside the fruit. On ringed-and-defoliated branches results differ in degree but are similar in nature. Thus the total solids lost by either the fleshy pericarp alone or the stony pericarp alone are sufficient to equal the gain in the embryo. Likewise the reducing substances from both fleshy and stony pericarp are more than sufficient, and the losses in sucrose also. In ether extract the fleshy pericarp alone could supply the material, even without resort to computations involving conversion of reducing substances or sucrose to glycerol trioleate. For protein, as on the check branches, the losses from the fleshy and stony pericarp are not sufficient to equal the gain by the embryo. Only when the losses by the twigs are included are sufficient nitrogenous materials secured to equal the gain by the embryo. Accordingly there can be no question but that the nitrogenous material, at least, comes from outside the fruit.

In the case of a fruit growing under natural environment, it is not possible to say whether materials mobilized in the embryo are derived directly from the endosperm, nucellus, integuments, stony pericarp, or fleshy pericarp and are in turn replaced from outside the fruit, or whether they are derived directly from outside the fruit. The data do not preclude the possibility that the embryo may develop, in part at least, from materials drawn directly from the fleshy pericarp, stony pericarp, endosperm, nucellus, and integuments. It is not necessary, however, to assume that materials which supply the embryo are derived from the fruit, but rather that materials move into both the fruit and the embryo in amounts more than sufficient to supply the embryo, and that when the supply is limited, as by ringing and defoliation, the materials may move from the fruit into the embryo. Such a situation probably occurs on trees low in vigor, or on trees heavily loaded with fruit, resulting in immature, low-quality fruit similar to fruit in these tests on branches which were ringed and defoliated.

MURNEEK (9) has discussed the importance of the developing embryo and has shown in *Cleome spinosa* that developing seeds were responsible for a reduction in growth of the female reproductive organs, thus bringing about sterility. The results of EWART (3, 4) with the pear are also of interest in this connection. He has shown that seedless fruits have a higher sugar content and lower acid content and ripen earlier than seeded fruits of the same variety, and that fruits with high seed content affect the nutritional condition of the tree. From this he has reasoned that trees bearing seedless fruits should be less depleted by fruit production than trees bearing highly seeded fruits. The data presented in this paper carry similar implications.

## Summary

1. The results deal with the development and chemical changes in twigs, fleshy pericarp, stony pericarp, integuments, nucellus, endosperm, and embryo of the Elberta and Ward Late varieties of peaches during the seasons of 1936 and 1937, following ringing and defoliation designed to check development of the fruit and to determine the effect upon development of the embryo. Morphological changes were noted, and determinations were made of total solids, reducing substances as glucose, sucrose, ether extract, and protein (nitrogen $\times 6.25$).

2. The data supplement the results in a previous paper which showed that it is abortion of the embryo which induces early-ripening of the fruit, and not the reverse, by demonstrating that when the supply of materials to the embryo from outside the fruit is limited, as by ringing and defoliation, the embryo continues to increase in size and storage materials continue to be mobilized. At the same time similar materials in the endosperm, nucellus, integuments, stony pericarp, fleshy pericarp, and adjacent twigs either decrease in amount or are retarded in mobilization.

3. The results are discussed with reference to drop fruits, movement of materials between the various tissues, and the physiological role which a fruit may play in the development of the embryo.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK

## LITERATURE CITED


1. BRADBURY, DOROTHY, A comparative study of the developing and aborting fruits of *Prunus cerasus*. Amer. Jour. Bot. **16**:525–542. 1929.
2. DETJEN, L. R., Physiological dropping of fruits. Delaware Agr. Exp. Sta. Bull. 143. 1926.
3. EWART, RICHARD, Der Einfluss des Kernes auf die Entwicklung der Frucht und auf die Ernährungsvorgänge in Baume. Jahresbericht des Königl. Pomologischen Institutes zu Proskau **1908**:95. 1910.
4. ———, Die Abhängigkeit des Reiseprozesses der Frucht von der Entwicklung der Samen. Jahresbericht des Königl. Pomologischen Institutes zu Proskau **1909**:110–111. 1910.
5. FINCH, A. H., and VAN HORN, C. W., The physiology and control of pecan nut filling and maturity. Arizona Agr. Exp. Sta. Tech. Bull. 62. 1936.
6. LLOYD, F. E., Development and nutrition of the embryo, seed and carpel in the date, *Phoenix dactylifera*. Missouri Bot. Gard. Ann. Rept. **21**:103–164. 1910.
7. LOTT, R. V., The growth rate and chemical composition of the Hiley peach from stone formation to flesh maturity. Proc. Amer. Soc. Hort. Sci. **29**:1–7. 1933.
8. ———, Further studies of peach fruit growth. Proc. Amer. Soc. Hort. Sci. **30**:201–202. 1934.
9. MURNEEK, A. E., Growth and development as influenced by fruit and seed formation. Plant Physiol. **7**:79–90. 1932.
10. SHUHART, D. V., Endosperm and embryo development as related to filling of pecans and walnuts. Proc. Amer. Soc. Hort. Sci. **28**:161–163. 1935.
11. THOR, C. J. B., and SMITH, C. L., A physiological study of seasonal changes in the composition of the pecan during fruit development. Jour. Agr. Res. **50**:97–121. 1935.
12. TUKEY, H. B., Embryo abortion in early ripening varieties of *Prunus avium*. BOT. GAZ. **44**:433–468. 1933.
13. ———, Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. **30**:209–218. 1933.
14. ———, Development of cherry and peach fruits as affected by destruction of the embryo. BOT. GAZ. **98**:1–24. 1936.
15. ———, Growth patterns of plants developed from immature embryos in artificial culture. BOT. GAZ. **99**:630–665. 1938.
16. VALLEE, M. C., Sur la presence du saccharose dans les amondes et sur son rôle dans la formation de l'huile. Compt. Rend. Acad. Sci. (Paris) **136**: 114–117. 1903.

# CYTOLOGICAL INVESTIGATIONS IN DESMODIUM AND LESPEDEZA

J. ORAN YOUNG

(WITH FORTY-THREE FIGURES)

## Introduction

The genera *Desmodium* Desv. and *Lespedeza* Michx. are closely allied taxonomically. They are placed by TAUBERT (12) in the subtribe Desmodiinae, tribe Hedysareae, subfamily Papilionatae of the family Leguminosae. Certain species have been variously assigned to either genus by different workers. At present, however, a fairly constant classification of the species naturally occurring in this country is to be found in the principal floras. Divergences of opinion are chiefly concerned with the specific or subspecific rank of certain forms.

According to TAUBERT, *Desmodium* includes some 160 species, but current investigations indicate that the number is much greater. The genus is widely distributed in both hemispheres, chiefly in North America, southern South America, eastern Asia, subtropical Australia, and South Africa. About forty species are indigenous to the United States but the greatest number of species is found in Mexico. The haploid number of chromosomes has been reported as eleven for four species: *D. perpesium* DC. (6), *D. grandiflorum* (Walt.) DC. (1), *D. canadense* (L.) DC., and *D. tortuosum* (Sw.) DC. (11).

In a recent paper PIERCE (8) gives brief descriptions of the morphology of the somatic chromosomes for twenty-five species and varieties of *Lespedeza*, and summarizes the results of earlier cytological work on the genus. The counts recorded in PIERCE's paper are included in table 2.

## Material and methods

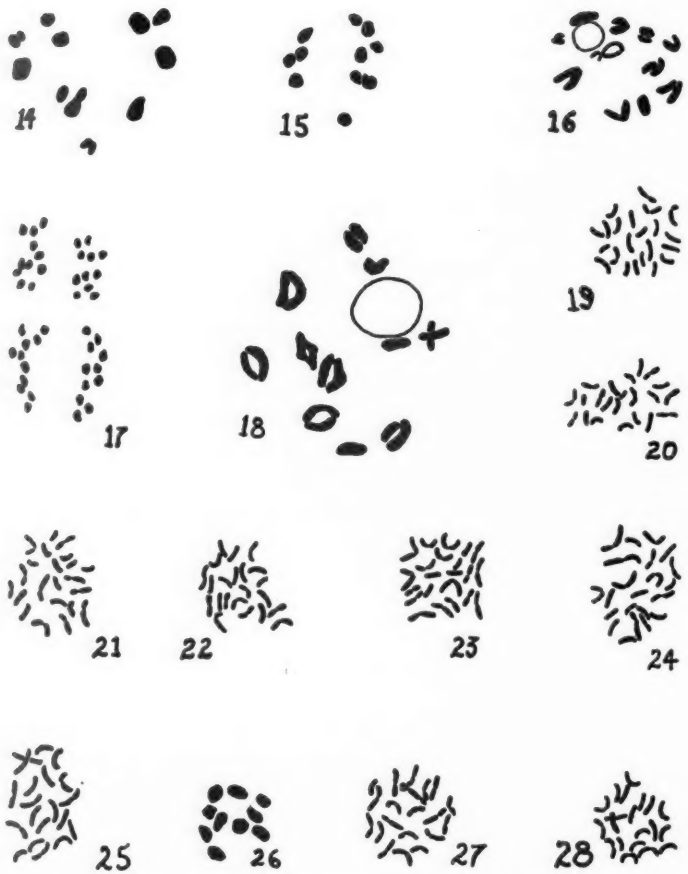
Most of the material for the present paper was collected along the Atlantic coastal plain and adjacent highland areas, from New Hamp-

shire to southern peninsular Florida, during the summers of 1938 and 1939. Additional material was obtained north of Lake Seneca in west-central New York state. Seeds of certain species were furnished by the Division of Forage Crops and Diseases, U.S. Department of Agriculture, through the kindness of Mr. Roland McKee. Seeds of *D. triquetrum* DC. were sent by Mr. José I. Otero of the Agricultural Experiment Station, Rio Piedras, Puerto Rico. Mr. Walter V. Brown collected flower buds of three species in New Hampshire. The writer expresses his thanks for these contributions.

Determinations of haploid chromosome numbers were made by the use of iron-aceto-carmine smears. Flower buds were fixed in a mixture of two parts absolute alcohol and one part glacial acetic acid. After 2–5 days the fixative was replaced by 80 per cent alcohol, in which the material was held until the smears were prepared. Difficulty in securing satisfactory differentiation between chromosomes and cytoplasm proved to be a serious handicap. Treatment of the flower buds before smearing with a few drops of hydrogen peroxide in 50 cc. of 80 per cent alcohol or heating the smears at 95° C. for as long as 15 minutes was often of distinct aid in securing better contrast. All figures of meiotic chromosomes are shown at a magnification of about 1100. These were made with the aid of a camera lucida using a 10× ocular and 1.8 mm. apochromatic objective.

For counting the diploid numbers, tips were taken from secondary roots of plants grown in the greenhouse or from primary roots of young seedlings grown on moist blotting paper. On the whole, clearer figures were obtained from secondary roots, but the larger cells of the young primary roots permitted better separation of the chromosomes in some instances. Scarification of the seed coats or exposing the seeds to sub-freezing temperatures usually increased the percentage and rapidity of germination. Figures 4, 6, 19, 25, 28, 32, 36, and 42 are from secondary roots fixed in Navashin's fluid. These figures, at a magnification of approximately 2300×, were obtained by using a 15× ocular and 1.8 mm. apochromatic objective, with the aid of a camera lucida. All other root tip metaphases are from primary roots fixed in La Cour's 2BE. They were drawn with the aid of a camera lucida using a 15× compensating ocular and a 90× apochromatic objective, n.a. 1.40, with a yellow green filter. La Cour's

FIGS. 1–13.—Fig. 1, *D. nudiflorum*, diakinesis. Fig. 2, *D. grandiflorum*, diakinesis. Fig. 3, *D. rotundifolium*, metaphase I. Fig. 4, *D. glabellum*, root tip metaphase. Fig. 5, *D. strictum*, metaphase I. Fig. 6, *D. canescens*, root tip metaphase. Fig. 7, same, diakinesis. Fig. 8, *D. paniculatum*, anaphase I. Fig. 9, *D. dillenii*, metaphase I. Fig. 10, *D. bracteosum*, diakinesis. Fig. 11, *D. rhombifolium*, root tip metaphase. Fig. 12, *D. laevigatum*, metaphase I. Fig. 13, *D. viridiflorum*, late diakinesis. Nucleoli in outline.




FIGS. 14–28.—Fig. 14, *D. canadense*, metaphase I. Fig. 15, *D. rigidum*, metaphase I. Fig. 16, *D. obtusum*, diakinesis. Fig. 17, *D. marilandicum*, anaphase II. Fig. 18, *D. tortuosum*, diakinesis. Fig. 19, *D. incanum*, root tip metaphase. Fig. 20, *D. triquetrum*, root tip metaphase. Fig. 21, *D. latifolium*, root tip metaphase. Fig. 22, *D. discolor*, root tip metaphase. Fig. 23, *D. gyrans*, root tip metaphase. Fig. 24, *D. gyroides*, root tip metaphase. Fig. 25, *L. repens*, root tip metaphase. Fig. 26, same, metaphase I. Fig. 27, *L. procumbens*, root tip metaphase. Fig. 28, *L. violacea*, root tip metaphase. Nucleoli in outline.

2BE fixative produced a slight shrinkage of the chromosomes but gave better definition of the constrictions than could be obtained after Navashin's fluid.

Whenever possible, herbarium specimens have been prepared to accompany the cytological record. In the case of seeds provided by other persons, the writer has accepted their identification but used the nomenclature retained by Index Kewensis.

## Observations

### Desmodium

The species investigated, together with the chromosome numbers and sources of material (or collection numbers if seed was obtained from the U.S. Department of Agriculture), are listed in table 1. The haploid number of chromosomes is eleven in all species studied.

While no attempt has been made to work out the karyotypes of the various species, certain morphological features are apparent. The chromosomes, as seen in somatic metaphases, vary in length from about 1 to 3 $\mu$. Median constrictions appear to be most frequent, commonly occurring where there is a bend of the chromosomes. Submedian and subterminal constrictions also occur, probably in all species observed. Chromosomes which carry small terminal bodies are interpreted as having subterminal constrictions rather than true satellites.

### Lespedeza

The chromosome counts determined in the present investigation, as well as those of the earlier workers in the genus, are listed in table 2. The morphology of the chromosomes is very similar to that in *Desmodium*. The chromosomes of *L. striata* (fig. 42), however, are decidedly smaller than those of other species observed. In three species, *L. procumbens*, *L. violacea*, and *L. frutescens* (fig. 33), it was possible to observe only nine bivalents in smear preparations of microspore mother cells, although root tip metaphases clearly showed twenty chromosomes. It is possible that the tenth pair of chromosomes was obscured by the nucleolus, which is apparently present until metaphase I or later.

Since the earliest chromosome counts made by the writer for *L. virginica* differed from that recorded by Pierce (**8**), many observations were made on meiotic material as well as on root tips

fixed in both Navashin's fluid and La Cour's 2BE. In all, about twenty drawings were made from eight different roots. The clearest

TABLE 1

CHROMOSOME NUMBERS IN DESMODIUM SPECIES STUDIED

| SPECIES | FIGURE | SOURCE OF MATERIAL | N | 2N |
|---|---|---|---|---|
| D. nudiflorum (L.) DC. | 1 | Wyalusing, Pa. | 11 | ..... |
| D. nudiflorum (L.) DC. | ..... | Wilton, Conn. | 11 | ..... |
| D. grandiflorum (Walt.) DC. | 2 | Redding, Conn. | 11 | ..... |
| D. grandiflorum (Walt.) DC. | ..... | Ossipee, N.H. | 11 | ..... |
| D. grandiflorum (Walt.) DC. | ..... | Wyalusing, Pa. | 11 | ..... |
| D. rotundifolium (Michx.) DC. | 3 | Junius, N.Y. | 11 | ..... |
| D. rotundifolium (Michx.) DC. | ..... | Wilton, Conn. | 11 | ..... |
| D. glabellum (Michx.) DC. | 4 | Wilton, Conn. | ..... | 22 |
| D. strictum (Pursh) DC. | 5 | McBee, S.C. | 11 | ..... |
| D. canescens (L.) DC. | 6 | Easton, Conn. | ..... | 22 |
| D. canescens (L.) DC. | 7 | Wake Forest, N.C. | 11 | ..... |
| D. paniculatum (L.) DC. | 8 | Wilton, Conn. | 11 | ..... |
| D. paniculatum (L.) DC. | ..... | Natural Bridge, Va. | 11 | ..... |
| D. paniculatum (L.) DC. | ..... | Junius, N.Y. | 11 | ..... |
| D. dillenii Darl. | 9 | Wake Forest, N.C. | 11 | ..... |
| D. dillenii Darl. | ..... | Wilton, Conn. | 11 | ..... |
| D. dillenii Darl. | ..... | Swedesboro, N.J. | 11 | ..... |
| D. bracteosum (Michx.) DC. | 10 | Swedesboro, N.J. | 11 | ..... |
| D. bracteosum (Michx.) DC. | ..... | Redding, Conn. | 11 | ..... |
| D. rhombifolium (Ell.) DC. | 11 | Tavares, Fla. | ..... | 22 |
| D. laevigatum (Nutt.) DC. | 12 | Wake Forest, N.C. | 11 | ..... |
| D. laevigatum (Nutt.) DC. | ..... | McKenny, Va. | 11 | ..... |
| D. viridiflorum (L.) Beck | 13 | Swedesboro, N.J. | 11 | ..... |
| D. viridiflorum (L.) Beck | ..... | Sumter, S.C. | 11 | ..... |
| D. canadense (L.) DC. | 14 | Nesquehoning, Pa. | 11 | ..... |
| D. canadense (L.) DC. | ..... | Ossipee, N.H. | 11 | ..... |
| D. rigidum (Ell.) DC. | 15 | Wilton, Conn. | 11 | ..... |
| D. rigidum (Ell.) DC. | ..... | Estelle Manor, N.J. | 11 | ..... |
| D. obtusum (Muhl.) DC. | 16 | Swedesboro, N.J. | 11 | ..... |
| D. obtusum (Muhl.) DC. | ..... | Naranja, Fla. | 11 | ..... |
| D. marilandicum (L.) DC. | 17 | Swedesboro, N.J. | 11 | ..... |
| D. marilandicum (L.) DC. | ..... | Junius, N.Y. | 11 | ..... |
| D. tortuosum (Sw.) DC. | 18 | Jacksonville, Fla. | 11 | ..... |
| D. tortuosum (Sw.) DC. | ..... | Miami, Fla. | 11 | ..... |
| D. incanum (Sw.) DC. | 19 | Bunnell, Fla. | ..... | 22 |
| D. triquetrum DC. | 20 | ..... | ..... | 22 |
| D. latifolium DC. (=D. lasiocarpum Kuntze) | 21 | FPI 103618 | ..... | 22 |
| D. discolor Vog. | 22 | FPI 133429 | ..... | 22 |
| D. gyrans DC. | 23 | FPI 64038 | ..... | 22 |
| D. gyroides DC. | 24 | FPI 77297 | ..... | 22 |

figures appeared definitely to show twenty chromosomes as the diploid number. It should be noted, however, that it is sometimes difficult to distinguish between one large chromosome with a median constriction and two smaller ones lying end to end.

## TABLE 2

### CHROMOSOME NUMBERS IN LESPEDEZA

| SPECIES | FIGURE | SOURCE OF MATERIAL | N | 2N | INVESTIGATOR |
|---|---|---|---|---|---|
| Section I. Archilespedeza Taub. | | | | | |
| A. Macrolespedeza Maxim. | | | | | |
| L. bicolor Turcz. | .... | ..... | 9 | ..... | Kawakami |
| L. bicolor Turcz. | .... | 81644 | .... | 22 | Pierce |
| L. cyrtobotrya Miq. | .... | ..... | 9 | ..... | Kawakami |
| L. cyrtobotrya Miq. | .... | FPI 82092 | .... | 22 | Pierce |
| L. cyrtobotrya var. pedunculata Nakai | .... | FPI 104066 | .... | 22 | Pierce |
| B. Eulespedeza Maxim. | | | | | |
| L. repens Bart. | .... | F.C. 21051 | .... | 20 | Pierce |
| L. repens Bart. | 25 | Wilton, Conn. | .... | 20 | Young |
| L. repens Bart. | 26 | Estelle Manor, N.J. | 10 | ..... | Young |
| L. repens Bart. | .... | Roberta, Ga. | 10 | ..... | Young |
| L. procumbens Michx. | .... | F.C. 85228 | .... | 20 | Pierce |
| L. procumbens Michx. | 27 | Wilton, Conn. | .... | 20 | Young |
| L. procumbens Michx. | .... | Sylva, N.C. | 9* | ..... | Young |
| L. procumbens Michx. | .... | Estelle Manor, N.J. | 9* | ..... | Young |
| L. violacea Pers. | .... | ..... | .... | 20 | Pierce |
| L. violacea Pers. | 28 | Wilton, Conn. | 9* | 20 | Young |
| L. nuttallii Darl. | 29 | Junius, N.Y. | 10 | ..... | Young |
| L. nuttallii Darl. | .... | Sylva, N.C. | 10 | ..... | Young |
| L. nuttallii Darl. | .... | Estelle Manor, N.J. | 10 | ..... | Young |
| L. stuvei Nutt. | 30 | Estelle Manor, N.J. | 10 | ..... | Young |
| L. stuvei Nutt. | .... | Sumter, S.C. | 10 | ..... | Young |
| L. stuvei Nutt. | 31 | Tifton, Ga. | 10 | ..... | Young |
| L. stuvei Nutt. | .... | Sag Harbor, L.I. | 10 | ..... | Young |
| L. stuvei Nutt. | .... | ..... | .... | 20 | Pierce |
| L. floribunda Bge. | .... | 90996 | .... | 22 | Pierce |
| L. virgata DC. | .... | FPI 90167 | .... | 22 | Pierce |
| L. frutescens (L.) Britton | 32 | Wilton, Conn. | .... | 20 | Young |
| L. frutescens (L.) Britton | 33 | Redding, Conn. | 9* | ..... | Young |
| L. virginica (L.) Britton | 34 | Sumter, S.C. | 10 | ..... | Young |
| L. virginica (L.) Britton | .... | Estelle Manor, N.J. | 10 | ..... | Young |
| L. virginica (L.) Britton | .... | Wilton, Conn. | 10 | ..... | Young |
| L. virginica (L.) Britton | 35 | Central Falls, R.I. | .... | 20 | Young |
| L. virginica (L.) Britton | .... | F.C. 21040 | .... | 20 | Young |
| L. virginica (L.) Britton | .... | ..... | .... | 22 | Pierce |
| L. simulata Mackenzie & Bush | 36 | Estelle Manor, N.J. | .... | 20 | Young |
| L. capitata Michx. | 37 | Center Ossipee, N.H. | 10 | ..... | Young |
| L. capitata Michx. | .... | Wilton, Conn. | 10 | ..... | Young |
| L. capitata Michx. | .... | ..... | .... | 20 | Pierce |
| L. capitata var. velutina (Bicknell) Fernald | 38 | Estelle Manor, N.J. | .... | 20 | Young |
| L. hirta (L.) Hornem. | 39 | Junius, N.Y. | 10 | ..... | Young |
| L. hirta (L.) Hornem. | .... | Roberta, Ga. | 10 | ..... | Young |
| L. hirta (L.) Hornem. | .... | Wilton, Conn. | 10 | ..... | Young |
| L. hirta (L.) Hornem. | .... | 21069 | .... | 20 | Pierce |
| L. augustifolia (Pursh) Ell. | 40 | Sumter, S.C. | 10 | ..... | Young |
| L. sericea Benth. | .... | ..... | .... | 18 | Cooper |
| L. sericea Benth. | .... | ..... | 9 | ..... | Pierce |
| L. variegata Cambess. | .... | F.C. 21866 | .... | 18 | Cooper |
| L. variegata Cambess. | .... | F.C. 21866 | .... | 20 | Pierce |
| L. pilosa Maxim. | .... | F.C. 12086 | .... | 20 | Pierce |
| L. tomentosa Siebold | .... | ..... | .... | 20 | Cooper |

* Number observed.

TABLE 2—*Continued*

| SPECIES | FIGURE | SOURCE OF MATERIAL | N | 2N | INVESTIGATOR |
|---|---|---|---|---|---|
| Section II. Campylotropsis Bge. | | | | | |
| L. macrocarpa Bge. | .... | F.C. 90998 | .... | 22 | Pierce |
| Section III. Microlespedeza Maxim. | | | | | |
| L. striata var. Kobe | .... | F.C. 22242 | .... | 22 | Pierce |
| L. striata (Thunb.) H. & A. | 42 | F.C. 22590 | .... | 22 | Young |
| L. stipulacea Maxim. | .... | ..... | .... | 20 | Cooper |
| Section undetermined | | | | | |
| L. latissima Nakai | .... | F.C. 19283 | .... | 20 | Pierce |
| L. inshanica (Maxim.) Schind. | .... | FPI 88315 | .... | 20 | Pierce |
| L. maximowiczii Gandog. | .... | 82482 | .... | 22 | Pierce |
| L. thunbergii Nakai | .... | FPI 25009 | .... | 22 | Pierce |
| L. thunbergii Nakai | 41 | Wilton, Conn. | 11 | ..... | Young |
| L. uekii Nakai | 43 | FPI 108231 | .... | 22 | Young |
| L. japonica var. intermedia Nakai | .... | FPI 82094 | .... | 22 | Pierce |
| L. robusta Nakai | .... | FPI 108229 | .... | 22 | Pierce |
| L. homoloba Nakai | .... | ..... | 9 | ..... | Kawakami |
| L. sieboldi Miq. (=L. thunbergii Nakai) | .... | ..... | 9 | ..... | Kawakami |
| L. sieboldi var. albiflora Schneid. | .... | ..... | 9 | ..... | Kawakami |
| L. daurica Schind. | .... | F.C. 89740 | .... | 36 | Cooper |
| L. daurica Schind. | .... | F.C. 89740 | .... | *ca.* 44 | Pierce |
| L. daurica var. shimadai Masam. and Hosokawa | .... | FPI 90353 | .... | *ca.* 44 | Pierce |

Specimens referred to *L. nuttallii* from Junius, New York, and Sylva, North Carolina, exhibited the leaf shape and peduncle length of *L. hirta*, together with pubescence characters suggestive of *L. frutescens*. Mature fruiting specimens could not be obtained, but the calyx in both cases resembled that of typical *L. nuttallii* collected in southern New Jersey, where the species is not uncommon. The pollen grains of this material showed an unusual size variation, the smallest having a diameter slightly more than one-half that of the largest.

Specimens referred to *L. stuvei* from Tifton, Georgia, and Sag Harbor, Long Island, New York, are apparently intermediate between this species and *L. nuttallii*. Examination revealed ten bivalents at diakinesis and a notable size variation of the pollen grains. A white form of *L. stuvei* also showed ten bivalents at diakinesis.

Young buds of *L. capitata* var. *velutina* were fixed at Sag Harbor

FIGS. 29–43.—Fig. 29, *L. nuttallii*, diakinesis. Fig. 30, *L. stuvei*, diakinesis. Fig. 31, same (a form resembling *L. nuttallii*), diakinesis. Fig. 32, *L. frutescens*, root tip metaphase. Fig. 33, same, diakinesis, showing only nine bivalents. Fig. 34, *L. virginica*, metaphase I. Fig. 35, same, root tip metaphase. Fig. 36, *L. simulata*, root tip metaphase. Fig. 37, *L. capitata*, metaphase I. Fig. 38, *L. capitata* var. *velutina*, root tip metaphase. Fig. 39, *L. hirta*, metaphase I. Fig. 40, *L. augustifolia*, metaphase I. Fig. 41, *L. thunbergii*, diakinesis. Fig. 42, *L. striata*, root tip metaphase. Fig. 43, *L. uekii*, root tip metaphase. Nucleoli in outline.

and at Smithfield, New Jersey. In material from both sources the anthers had failed to reach full size, evidently withering before anthesis. Pollen grains were small, having an average diameter about two-thirds that of typical pollen grains from *L. capitata*. No meiotic figures could be obtained either in smears or in sectioned material, but the small pollen grains appeared to contain both a vegetative and a generative nucleus. Root tip metaphases showed the diploid number to be twenty-two.

## Discussion

### Desmodium

The chromosome number tabulations of Gaiser (**2, 3, 4, 5**) show that certain families and genera are particularly rich in polyploid species, while others are nearly all, or entirely, diploid. Tischler (**13**) cites the Polygonaceae, Rosaceae, Malvaceae, and Rubiaceae as highly polyploid families, while the Leguminosae and Umbelliferae are families poor in polyploidy. Senn (**11**) lists forty-two completely diploid genera in the Leguminosae and only ten that are entirely polyploid. In eighteen other genera of the Leguminosae more than half of the species are diploid. Only 23 per cent of the species so far reported in the family are polyploid. When the family is considered in its entirety, it is not surprising to find the genus *Desmodium* completely diploid.

Total absence or very low percentage of polyploidy has been cited for many genera. Woodworth (**17**) and Wetzel (**14, 15**) list fifteen species and varieties of *Corylus* as diploid, and no polyploidy has been reported; O'Mara (**7**) cites five species and ten varieties in the genus *Forsythia* as diploid; Roscoe (**9**) finds eight diploids in *Wistaria;* Sax and Kribs (**10**) describe eleven diploid species in six sections of the genus *Viburnum;* and Senn (**11**) finds forty-one diploids in *Lathyrus* as against one polyploid.

### Lespedeza

Many taxonomists have reported hybrids in the genus and some have been considered of sufficient constancy to receive a place in the floras. Certain intermediate forms that have been examined, however, have not exhibited meiotic irregularity or unusual chromosome numbers. Even though ten species and two varieties of *Lespe-*

*deza*—as well as certain intermediate forms—were found growing in an area about an acre in extent at Estelle Manor, New Jersey, in none of this material were any unusual karyological conditions noted.

A plant collected at Junius, New York, was identified as *L. nuttallii* Darl. WIEGAND and EAMES (**16**), however, consider forms like *L. nuttallii* found in that region to be hybrids. They state, "Forms occur . . . . which agree more or less closely with *L. nuttallii* Darl. and with *L. stuvei* Nutt. These specimens do not constitute distinct categories but present various combinations of the characters of *L. intermedia* Wats. [=*L. frutescens* (L.) Britton] and *L. hirta*. . . . . In this region, therefore, the so-called *L. nuttallii* and *L. stuvei* are to be considered hybrids." The specimen collected by the writer at Junius, and a similar one from Sylva, North Carolina, showed ten bivalents and no irregularity other than size variation of the pollen grains. This condition may be related to genetic factors.

If hybridization does occur in *Lespedeza* it is apparently attended by complete compatibility of the parental chromosomes. This hypothesis could be substantiated only by cytological observations on experimentally induced hybrids.

## Summary

1. The haploid number eleven is reported for fifteen species in *Desmodium*. The diploid number twenty-two is reported for nine species.

2. Gametic numbers observed in *Lespedeza* are eleven for one species, ten for seven species, and nine for three species. Where the haploid number nine is reported, the presence of twenty chromosomes in root tip metaphases indicates that the true gametic number is ten. Diploid numbers reported are twenty for six species and one variety, and twenty-two for two species.

3. In both genera the chromosomes vary in length, from approximately 1 to 3 $\mu$.

4. Pollen grains showed size variation in possibly hybrid species of *Lespedeza*, but no karyological abnormalities were noted.

5. The anthers of *L. capitata* var. *velutina* had withered before anthesis in material from two widely separated localities and only a few unusually small pollen grains were found.

and at Smithfield, New Jersey. In material from both sources the anthers had failed to reach full size, evidently withering before anthesis. Pollen grains were small, having an average diameter about two-thirds that of typical pollen grains from *L. capitata*. No meiotic figures could be obtained either in smears or in sectioned material, but the small pollen grains appeared to contain both a vegetative and a generative nucleus. Root tip metaphases showed the diploid number to be twenty-two.

## Discussion

### Desmodium

The chromosome number tabulations of Gaiser (**2, 3, 4, 5**) show that certain families and genera are particularly rich in polyploid species, while others are nearly all, or entirely, diploid. Tischler (**13**) cites the Polygonaceae, Rosaceae, Malvaceae, and Rubiaceae as highly polyploid families, while the Leguminosae and Umbelliferae are families poor in polyploidy. Senn (**11**) lists forty-two completely diploid genera in the Leguminosae and only ten that are entirely polyploid. In eighteen other genera of the Leguminosae more than half of the species are diploid. Only 23 per cent of the species so far reported in the family are polyploid. When the family is considered in its entirety, it is not surprising to find the genus *Desmodium* completely diploid.

Total absence or very low percentage of polyploidy has been cited for many genera. Woodworth (**17**) and Wetzel (**14, 15**) list fifteen species and varieties of *Corylus* as diploid, and no polyploidy has been reported; O'Mara (**7**) cites five species and ten varieties in the genus *Forsythia* as diploid; Roscoe (**9**) finds eight diploids in *Wistaria;* Sax and Kribs (**10**) describe eleven diploid species in six sections of the genus *Viburnum;* and Senn (**11**) finds forty-one diploids in *Lathyrus* as against one polyploid.

### Lespedeza

Many taxonomists have reported hybrids in the genus and some have been considered of sufficient constancy to receive a place in the floras. Certain intermediate forms that have been examined, however, have not exhibited meiotic irregularity or unusual chromosome numbers. Even though ten species and two varieties of *Lespe-*

*deza*—as well as certain intermediate forms—were found growing in an area about an acre in extent at Estelle Manor, New Jersey, in none of this material were any unusual karyological conditions noted.

A plant collected at Junius, New York, was identified as *L. nuttallii* Darl. WIEGAND and EAMES (**16**), however, consider forms like *L. nuttallii* found in that region to be hybrids. They state, "Forms occur . . . . which agree more or less closely with *L. nuttallii* Darl. and with *L. stuvei* Nutt. These specimens do not constitute distinct categories but present various combinations of the characters of *L. intermedia* Wats. [=*L. frutescens* (L.) Britton] and *L. hirta*. . . . . In this region, therefore, the so-called *L. nuttallii* and *L. stuvei* are to be considered hybrids." The specimen collected by the writer at Junius, and a similar one from Sylva, North Carolina, showed ten bivalents and no irregularity other than size variation of the pollen grains. This condition may be related to genetic factors.

If hybridization does occur in *Lespedeza* it is apparently attended by complete compatibility of the parental chromosomes. This hypothesis could be substantiated only by cytological observations on experimentally induced hybrids.

## Summary

1. The haploid number eleven is reported for fifteen species in *Desmodium*. The diploid number twenty-two is reported for nine species.

2. Gametic numbers observed in *Lespedeza* are eleven for one species, ten for seven species, and nine for three species. Where the haploid number nine is reported, the presence of twenty chromosomes in root tip metaphases indicates that the true gametic number is ten. Diploid numbers reported are twenty for six species and one variety, and twenty-two for two species.

3. In both genera the chromosomes vary in length, from approximately 1 to 3 $\mu$.

4. Pollen grains showed size variation in possibly hybrid species of *Lespedeza*, but no karyological abnormalities were noted.

5. The anthers of *L. capitata* var. *velutina* had withered before anthesis in material from two widely separated localities and only a few unusually small pollen grains were found.

The writer wishes to express his thanks to Professor G. L. CHURCH of Brown University for his generous aid during the course of the problem and to Professor J. M. BEAL for aid in certain critical determinations.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

## LITERATURE CITED

1. COOPER, D. C., Chromosome numbers in the Leguminosae. Amer. Jour. Bot. **23**:231–233. 1936.
2. GAISER, L. O., A list of chromosome numbers in angiosperms. Genetica **8**:401–484. 1926.
3. ———, Chromosome numbers in angiosperms. II. Bibliographia Genetica **6**:171–466. 1930.
4. ———, Chromosome numbers in angiosperms. III. Genetica **12**:161–260. 1930.
5. ———, Chromosome numbers in angiosperms. IV. Bibliographia Genetica **10**:105–250. 1933.
6. KAWAKAMI, J., Chromosome numbers in Leguminosae. Bot. Mag. Tokyo **44**:319–328. 1930.
7. O'MARA, J., Chromosome numbers in the genus *Forsythia*. Jour. Arnold Arboretum **11**:14–15. 1930.
8. PIERCE, W. P., Cytology of the genus *Lespedeza*. Amer. Jour. Bot. **26**:736–744. 1939.
9. ROSCOE, M., Cytological studies in the genus *Wistaria*. BOT. GAZ. **84**:171–186. 1927.
10. SAX, K., and KRIBS, D. A., Chromosomes and phylogeny in Caprifoliaceae. Jour. Arnold Arboretum **11**:147–153. 1930.
11. SENN, H. A., Chromosome number relationships in the Leguminosae. Bibliographia Genetica **12**:175–336. 1938.
12. TAUBERT, P., Leguminosae, In ENGLER and PRANTL, Die Natürlichen Pflanzenfamilien. Teil 3, Abt. 3. pp. 70–385. Leipzig. 1894.
13. TISCHLER, G., Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. Bot. Jahrb. **67**:1–36. 1935.
14. WETZEL, G., Chromosomen Zahlen bei den Fagales. Ber. Deutsch. Bot. Ges. **45**:251–252. 1927.
15. ———, Chromosomenstudien bei den Fagales. Ber. Deutsch. Bot. Ges. **46**:212–214. 1928.
16. WIEGAND, K. M., and EAMES, A. J., Flora of the Cayuga Lake basin, New York. Ithaca. 1926.
17. WOODWORTH, R. H., Cytological studies in the Betulaceae. II. *Corylus* and *Alnus*. BOT. GAZ. **88**:383–399. 1929.

# SEX EXPRESSION IN WILLOWS

ERNEST C. SMITH

(WITH FOUR FIGURES)

## Introduction

While willows are commonly dioecious, variations from this condition have been observed from early times. LINNAEUS described a monoecious specimen and gave it a specific name, *Salix androgyna*. European literature abounds in mention of what until recently were regarded merely as freaks. PENZIG (**18**) lists the titles of nearly one hundred papers describing peculiarities of inflorescence in thirty-two species and seven hybrids of this genus. Thirteen papers describe androgynous or gynandrous forms of the European *Salix caprea* and nearly as many report the same conditions in *Salix cinerea*. In the United States reports have been less frequent. The most detailed and complete reports are those of BICKNELL (**3, 4**) in 1880 and 1881. TWEEDY (**26**) also described the condition in 1880.

More recently these abnormalities have been carefully studied with the purpose of discovering, if possible, the cause or causes of these variations from the usual dioecious character. HERIBERT-NILSSON (**15**) in Sweden, RAINIO (**19**) in Finland, and SCHAFFNER (**20, 21, 22, 23, 24**) in the United States have approached the subject from this angle.

The writer has met with these abnormal forms in nine species of Rocky Mountain willows, in only one of which has the condition been previously reported.

## Observations

1. *Salix lasiandra caudata* (Nutt.) Sudw. (*S. caudata* Heller var. *bryantiana* Ball.). *Salix* 305 E. C. S.—This shrub is located on the bank of the Cache la Poudre River at La Porte, Colorado, at an elevation of approximately 5000 feet. It is 5 m. in height and entirely normal in all its vegetative characters. It has been observed at different seasons in five consecutive years. Some of the aments contain only staminate flowers, some only pistillate; but more than

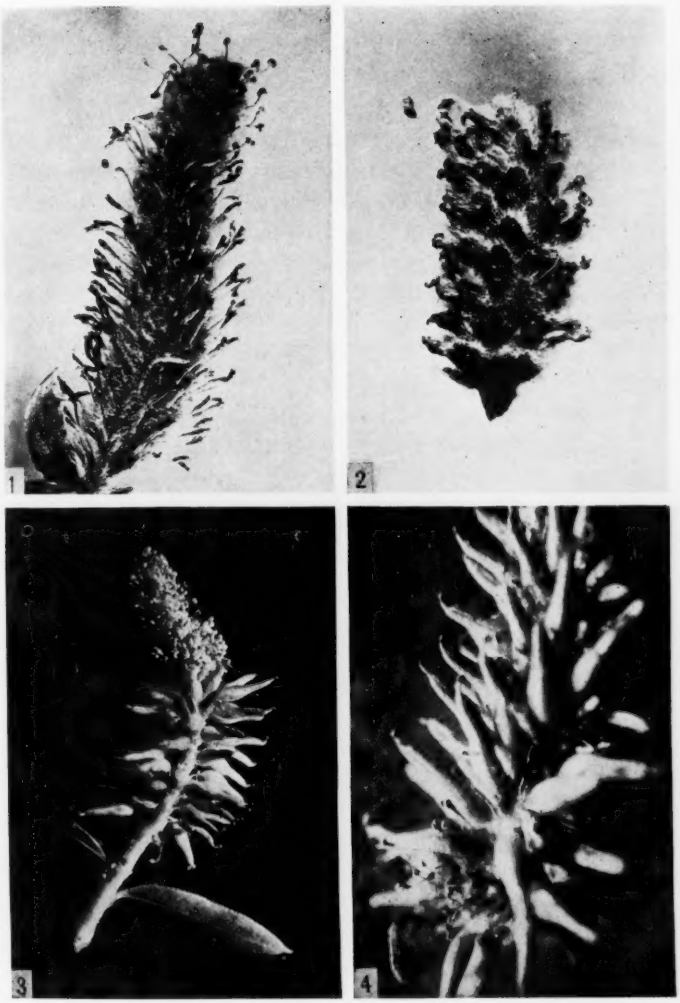
half contain both in varying proportions, the staminate flowers usually occurring at the tip of the ament. A twig taken at random, 3.5 dm. long, bore three aments, the upper two mixed, the lowest wholly staminate. Another twig, 4 dm. long, bore seven aments, three mixed and four pistillate. There are no abnormal flowers.

The same species as no. 305. *Salix* 955 E. C. S. (fig. 3).—This plant is located on the river bank about 2 miles above La Porte. It is a vigorous shrub about 4 m. high. Apparently it is basically staminate, more than half the aments being wholly of that type. As in no. 305, in the mixed aments the staminate flowers occur in the upper portion. The only difference observable is the presence of numerous perfect flowers at the base of the mixed aments (fig. 4). Again there are no abnormal stamens or pistils, only the unusual arrangement of these organs in the catkin. This plant was observed two consecutive years.

2. *Salix lasiandra* Benth., an unnumbered specimen collected by W. W. Jones near Bozeman, Montana, on May 25, 1901.—In the limited amount of material in hand the relative position of the staminate and pistillate flowers in the aments is less fixed than in the preceding individuals. In some aments the pistillate flowers are at the tip and in others the two kinds of flowers are so intermixed that it is only by careful dissection that one discovers there are no perfect flowers present. The stamens and pistils are normal.

3. *Salix scouleriana* Barratt. *Salix* 941 E. C. S.—The shrub is 2 m. tall, at the top of Rist Canyon, 20 miles west of Fort Collins, Colorado, at an elevation of approximately 7500 feet. The aments are pistillate, staminate, and mixed. In the mixed aments the staminate flowers are regularly at the tip. Abnormalities in individual stamens and pistils are sparingly present. Some stamen filaments bear swollen tips which resemble short ovaries and are tipped with stigmas. A few of these shortened ovaries have one lobe transformed into an anther. In a few other cases nonfunctional anthers sprout from between stigmas. Anthers normally glabrous present surfaces with the typical tomentose covering of normal ovaries.

4. *Salix planifolia* var. *nelsoni* Schn. (*Salix nelsoni* Ball).—A single shrub of this species, 2 m. high, was observed in Estes Park, Colorado, at an elevation of 7500 feet. It presented the monoecious character with mixed aments only, the staminate flowers at the tips.



FIGS. 1–4.—Fig. 1, ament of *Salix padophylla* showing stamens changing into pistils. Fig. 2, ament of *S. pseudolapponum* showing incipient change of pistils. Fig. 3, ament of *S. caudata* var. *bryantiana* with staminate tip and prevailingly pistillate base. Fig. 4, base of same ament showing perfect flowers (normal stamens and pistils in same flowers).

5. *Salix exigua* var. *stenophylla* (Rydb.) Schn. *Salix* 1008 E. C. S. —Here, instead of single individuals, are nine shrubs showing staminate, pistillate, and mixed aments. They are located near the bank of the Cache la Poudre River, not far from no. 955. Each shrub has from two to four stems averaging 2 m. tall. In all respects except the inflorescence they are indistinguishable from typical specimens, which are also present in large numbers. In the mixed aments the staminate flowers are usually massed at the base, the reverse of the situation in the preceding species, the division between the two kinds of flowers being well marked and abrupt. These plants have been observed for three consecutive years.

6. *Salix petrophila* Rydb. (*S. arctica* var. *petraea* Anders.). *Salix* 998 E. C. S.—Two specimens are growing on a steeply sloping bank above Iceberg Lake in Rocky Mountain National Park at an elevation of about 12,000 feet. Each individual bears two aments. Of these, three have the stamens at the tip, while the fourth has stamens, a few pistils, more stamens, and at the base several pistils. There are no abnormal stamens or pistils.

7. *Salix padophylla* Rydb. (*S. pseudomonticola* var. *padophylla* Ball). *Salix* 940 E. C. S.—A shrub 3.5 m. tall was observed on the bank of Fish Creek, Estes Park, Colorado, at an elevation of 7450 feet. Most of the catkins on this shrub are what HERIBERT-NILSSON (**15**) calls metamorphosans and RAINIO (**19**) calls intersexes (fig. 1). Apparently the plant is basically staminate, since it has functional stamens at the tips of a few aments and no functional pistils. The individual flowers exhibit graded stages of sex reversal. Stamens occur with the filaments distinct, but the anthers take on the appearance of shortened and shriveled ovaries. Below these are flowers with the filaments slightly united, then flowers with the filaments completely united. The ovaries formed at the tips of the filaments also display gradations of development, although stigmas are present in nearly all cases.

8. *Salix brachycarpa* Nutt. *Salix* 707 E. C. S.—A single specimen was found growing in moist ground near Poudre Lakes in Rocky Mountain National Park at an elevation of 10,750 feet. This individual also produces abnormal flowers of the type denominated intersexes. There are no normal flowers. Apparently the plant is

basically staminate but in process of transformation into the pistillate condition. Filaments occur, both distinct and united, but instead of anthers they bear at their tips small sacs or cups which are provided with stigmas. Occasionally anthers occur, but in unusual positions, almost sessile or attached to the side or tip of an abnormal ovary which is at the end of a long, filament-like pedicel. Some of the ovaries start near the base of the filaments, in which case they are slender, swelling out only near the tip. While the filaments of this species in normal specimens are glabrous and the ovaries densely tomentose, in these abnormal individuals curious displacements of the pubescence take place—the filaments or portions of them tomentose, the ovaries glabrous or tomentose only on one side. Where anther cells are most completely formed the connective is sometimes tomentose and prolonged some distance beyond the anthers. Under the binoculars the color contrast is striking. Where the union of the filaments is complete and the whole structure bears some resemblance to an ovary, the lower portion is yellow, the tip from purple to dark reddish brown.

9. *Salix pseudolapponum* von Seem. (*S. glaucops* Anders. of Manuals). *Salix* 1255B E. C. S.—A single specimen is growing in moist ground near Poudre Lake in the Rocky Mountain National Park, 7 dm. high. This specimen is of interest because it is the only one seen in which the sex reversal is from the pistillate to the staminate condition. Most of the aments bear normal pistillate flowers. A few bear some staminate flowers at the tip with intersexes where the two types meet. The clearly staminate flowers are not quite normal, the filaments showing the characteristic pubescence of the ovaries. The intersexes show ovaries with the stigmas changing to anthers (fig. 2).

## Discussion

While this last case is the only one observed by the writer showing a change in this direction, BICKNELL (**3, 4**) and TWEEDY (**26**) in this country and many investigators in Europe have reported this condition. RAINIO (**19**) found intersexes so common that he arranged them in five classes of androgynous and five classes of gynandrous forms, basing these upon the degree of change in the organs and on

what he conceived to be the basic condition from which the change started. It will be noticed that in five of the species described by the writer there are no abnormal flowers, the staminate and pistillate or perfect flowers all having normal stamens and pistils. Most reports specify intermediates as occurring at the transition between the two kinds of flowers.

These diverse forms of sex expression are by no means confined to willows. DARWIN (**8**), observing similar phenomena with a view to their significance for his theory of evolution, stated that "various hermaphrodite plants have become, or are becoming dioecious by many and exceedingly small steps." DAVEY and GIBSON (**9**) give detailed descriptions of dioecious, monoecious, and intermediate individuals of *Myrica gale*. DARLINGTON (**7**) lists many species showing these abnormalities, giving data taken from varied sources. SCHAFFNER (**20**) in his nearly thirty papers in this field includes one specifically dealing with willows.

The current theories of the cause of these abnormalities fall into two classes, one alleging that the causes are external to the plant, or at least have no relation to the segregation and combination of the units of the chromosomes, the other holding that the causes are for the most part internal and dependent upon the genes or upon these in combination with the action of the allosomes.

The most insistent and convincing advocate of the first theory is the late Dr. SCHAFFNER (**24**). By his experiments with hemp (**21**), Japanese hop (**22**), and mulberry (**20**) he was convinced that dioecious plants, whether staminate or pistillate, carried within themselves the potentialities of expression of the opposite sex (**23, 24**). He found nineteen out of one hundred individuals of *Salix amygdaloides* showing staminate, pistillate, and intermediate flowers, and applied the conclusions drawn from his experiments with other plants, stating that sex was purely physiological and in no way dependent upon segregation and combination of the units of chromosomes. His emphasis was upon external factors—light, temperature, and soil.

His contention that the potentialities for bisexual expression are present in willows and that external agents may and sometimes do cause sex reversal finds support in the observations of HARRISON (**14**), who stresses parasitism as an actual cause, stating without

qualification that androgyny in willows is due to castration by Eriophid mites. Distortion of organs due to insect deposition of eggs is a commonly observed fact; that an upset of balance of sex tendencies may be so caused is possible. While the writer has observed a few individual aments of willows in which this is a possible—but not certain—cause of sex reversal, recently direct evidence of modifications due to insect visitations has been evidenced by catkins which have fallen from a staminate specimen of Carolina poplar. Aments falling on May 23, after the leaves were well advanced, exhibited elongated axes and pedicels, forming loose inflorescences in which the disks were much thickened, the filaments very short, but the anthers still distinguishable as such. Aments falling on June 8 showed anthers no longer recognizable as such, partially transformed to ovaries, some of which were provided with stigmas. Sectioning showed the presence of numerous insects, identified at the Entomology Department of the College as mites.

These are not isolated cases. LOEHWING (**16**) cites numerous reports of similar modifications of sex expression, due not only to insects but also to the indirect action of fungi, which produce chemical substances which have a direct effect.

It must be concluded that willows and many dioecious plants do contain the potentialities for bisexual expression, and that sex reversal and other modifications may be and have been produced by a considerable variety of external agents.

This, however, does not rule out the action of the chromosomes in sex inheritance. SINNOTT and DUNN (**25**), bringing together data from many sources, state that a cytological basis for sex differences has been found in more than thirty dioecious plants. A statement taking cognizance of both sets of facts is that of ALLEN (**1**), who states, "There are two obvious facts; first, the potentiality for the production of any character that an organism under any possible condition can manifest must be represented in its hereditary constitution; and second, that this hereditary constitution provides only potentialities, whose expression or non-expression depends on the concurrence of environmental factors."

That some dioecious plants have had sex expression modified by external factors does not prove that these are the only agents which

can and do produce such changes, or that they are the actual agents which produce them under the conditions of natural occurrence. In the case of the willows observed by the writer the action of the external agents previously considered must be ruled out, because the normal and abnormal specimens were growing under identical ecological conditions.

SCHAFFNER takes the dioecious condition for granted and makes no attempt to account for it. If tendencies to expression of bisexuality are present in all individuals of the species, how explain the inhibition of one or the other expression in all but a few individuals? If dioecism is a climax character in certain groups of plants, as DARWIN (**8**) states and BESSEY (**2**) and many other American botanists believe, then this character is eventually impressed upon the germ plasm. HERIBERT-NILSSON posits an inhibiting factor regularly present in dioecious plants, its occasional absence setting free the tendency to bisexual expression.

As far as willows are concerned, HERIBERT-NILSSON provides conclusive evidence that hybridization is a definite cause of all the abnormalities of sex expression which have been previously described. In this connection RAINIO, who in two seasons of collecting found one hundred and forty individual willows which were abnormal, stated that in the district where he collected, hybrids were as abundant as pure lines. HÅKANSSON (**12, 13**) made cytological studies of some of HERIBERT-NILSSON'S abnormal hybrids and found that they were either triploid or polyploid. These results agree with the findings of BRIDGES (**6**) and DOBZHANSKY (**10**) in their investigations of *Drosophila*. They found that triploid intersexes begin as males, develop as such up to a certain point, after which they develop as females. This exactly describes the writer's no. 940 and several of the Swedish scientist's hybrids.

Hybridization is not the only cause of triploidy and polyploidy. DARLINGTON lists seventeen species of plants in which polyploidy has been observed in pure lines. HÅKANSSON reports its occurrence in *Salix aurita*. BLACKBURN and HARRISON (**5**) proved that at least two hybrid willows adhered to the dioecious condition, and that *Salix caprea*, one of the species used by HERIBERT-NILSSON in his hybridizing experiments, occasionally produced metamorphosans

when cultivated as a pure line. At least five of the specimens cited in this paper show no evidence of hybridization, the vegetative structures being true to specific type and the flowers with normal stamens and pistils. When associated with the perfect flowers one might consider the series as steps in a reversion to an earlier, more primitive type.

The mediating factors in the variations are undoubtedly the hormones. GOLDSCHMIDT (**11**) states, "Sex factors are substances which cause, take part in, or accelerate a reaction in proportion to the quantity present. The reaction itself is due to the presence of specific hormones of sexual differentiation." It is true that GOLDSCHMIDT worked primarily with insects and we are considering plants; but estrogenic materials have been found by WALKER and JANNEY (**27**) in the aments of willows. Whether in bisexual or dioecious plants, these hormones act at the points where the stamens or pistils are formed and usually act as completely segregating agents. Granting the presence of tendencies to both staminate and pistillate expression, it is probably the action of the hormones which is most responsive to the influence of external factors and led SCHAFFNER to declare that sex is physiologic rather than genetic.

But back of the action of hormones is always the genetic constitution of the individual, carrying all the potentialities which can find expression under any set of conditions. Apparently in willows there are several critical points for sex differentiation. Normally the form of expression is determined at an early stage and dioecious individuals appear; if the segregation comes later the monoecious condition is in evidence; and if still further delayed perfect flowers are formed. Evidently in the case of intermediates the segregation is delayed so long that the formation of normal stamens and pistils is no longer possible.

## Conclusions

1. Dioeciousness in willows probably expresses a terminal condition or climax in an evolutionary process which is impressed to a high degree on the germ plasm.

2. The potentialities for the expression of both sex tendencies are present in all individuals of the genus, but expression of one of

them is ordinarily in nature inhibited by the genetic constitution. Whether this is due to an inhibiting factor (**15**), to a realizator factor (**17**), or to a varying balance between genes and autosomes (**6**) is not yet clear.

3. Whatever accounts for the dioecious character, some of the variations from that condition are demonstrably due to external agents; others are just as demonstrably due to internal changes. Under the natural conditions in which willows occur, triploidy and polyploidy are common results of hybridization, but occur also in pure lines. In some cases, but not in all, these conditions bring about various abnormalities of sex expression.

4. Even though the underlying causes are in the germ plasm, the action at the point of segregation for stamens and pistils is that of the hormones, and that action may be modified by external factors.

BOTANY DEPARTMENT
COLORADO COLLEGE OF AGRICULTURE AND MECHANIC ARTS
FORT COLLINS, COLORADO

## LITERATURE CITED

1. ALLEN, C. E., Symposium on the biology of sex. New Orleans. 1931.
2. BESSEY, C. E., Evolution and classification. Proc. Amer. Assoc. Adv. Sci. **42**:237–251. 1893.
3. BICKNELL, E. F., Teratology. *Salix*. Bull. Torr. Bot. Club **7**:55–56. 1880.
4. ———, Stamens within the ovary of *Salix*. Bull. Torr. Bot. Club **8**:76–77. 1881.
5. BLACKBURN, K. B., and HARRISON, J. W. H., A preliminary account of the chromosomes and chromosome behavior in the Salicaceae. Ann. Bot. **38**: 361–378. 1924.
6. BRIDGES, C. B., Triploid intersexes in *Drosophila melanogaster*. Science **54**:252–254. 1921.
7. DARLINGTON, C. D., Recent advances in cytology. 1937.
8. DARWIN, CHARLES, The origin of species. 1859.
9. DAVEY, A. J., and GIBSON, C. M., Note on the distribution of sexes in *Myrica gale*. New Phytol. **16**:147–167. 1916.
10. DOBZHANSKY, TH., Genetical and environmental factors influencing the type of intersexes in *Drosophila melanogaster*. Amer. Nat. **64**: 1930.
11. GOLDSCHMIDT, R., The determination of sex. Nature **107**:78. 1921.
12. HÅKANSSON, A., Die Chromosomen in der Kreutzung *Salix viminalis*× *S. caprea* von Heribert-Nilsson. Hereditas **13**:1–52. 1921.
13. ———, Die Konjugation der Chromosomen bei einigen *Salix*-Bastarden. Hereditas **18**:199–214. 1934.

14. HARRISON, J. W. H., Sex in the Salicaceae and its modification by Eriophid mites and other insects. Brit. Jour. Exper. Biol. **1**:445–472. 1924.
15. HERIBERT-NILSSON, N., Experimentelle studien ueber Variabilitaet, Spaltung, Artbildung und Evolution in der Gattung *Salix*. Lunds. Univ. Arskr. **14**: No. 28, 1918.
16. LOEHWING, W. F., Physiological aspects of sex in angiosperms. Bot. Rev. **4**:581–625. 1938.
17. MARESQUELLE, H. J., Biologie generale. I. La signification de la difference. sexuelle. 1938.
18. PENZIG, O., Teratologie. 1922.
19. RAINIO, A. J., Ueber die Intersexualitaet bei der Gattung *Salix*. Ann. Zool. Botanicae Fennicae. (5) **2**:165–275. 1927.
20. SCHAFFNER, J. H., The nature of the dioecious condition in *Morus alba* and *Salix amygdaloides*. Ohio Jour. Sci. **19**:409–416. 1919.
21. ———, Complete reversal of sex in hemp. Science **50**:311–312. 1919.
22. ———, Sex reversal in Japanese hop. Bull. Torr. Bot. Club **50**:73–79. 1923.
23. ———, Sex determination and sex differentiation in the higher plants. Amer. Nat. **59**:115–127. 1925.
24. ———, Sex determination and sex differentiation in the light of observations and experiments on dioecious plants. Amer. Nat. **61**:319–332.
25. SINNOTT, E. W., and DUNN, L. C., Principles of genetics. 1939.
26. TWEEDY, FRANK, *Salix* and *Rubus*. Bull. Torr. Bot. Club **7**:56. 1880.
27. WALKER, B. S., and JANNEY, J. S., Estrogenic substances. II. An analysis of plant sources. Endocrin. **14**:389–392. 1930.

# DENSITY OF STOMATA AND OIL GLANDS AND INCIDENCE OF WATER SPOT IN THE RIND OF WASHINGTON NAVEL ORANGE[1]

F. M. TURRELL AND L. J. KLOTZ

(WITH NINE FIGURES)

## Introduction

In studies on water spot of the navel orange, FAWCETT, KLOTZ, and HAAS (2) observed that normal fruits with unscratched rinds, when immersed in distilled water for 16 hours, absorbed an average of 1.25 gm. water per fruit. They also noted that: "Water spot of navel oranges affects most severely three locations in the rind: that surrounding the navel, that adjacent to wounds, and the shoulder portion of the stem half" (2). Our observations had indicated that the orange epidermis contained stomata which might afford an entrance for water, and the present investigation was initiated to determine whether stomatal density in certain areas on the fruit surface was related to susceptibility to water spot.

The first symptom of water spot is the appearance of translucent edematous areas in the rind, which later become discolored. Since injection of orange oil into the albedo[2] causes similar discoloration, KLOTZ (4), and FAWCETT, KLOTZ, and HAAS (2) have attributed the discoloration to release of oil from the oil glands. Therefore oil-gland density on the rind surface has also been included in this study.

## Material and methods

Ten orange fruits were picked at random from each of four Washington Navel orange trees located in the plant pathology plots of the University of California Citrus Experiment Station at Riverside. Equatorial and polar diameters were measured with vernier calipers; and on this basis the fruits were separated into two groups, one of

[1] Paper no. 405, University of California Citrus Experiment Station.

[2] The term albedo is derived from the Latin *albus*, white. It has been in use in the citrus industry for some time and refers to the white inner portion of the rind of citrus fruits, differentiating it from the flavedo (yellow), oil-gland-containing outer portion.

large and one of small fruits. The oranges were wiped with cotton saturated with xylene or benzene, and were then similarly wiped with absolute ethanol. Two freehand sections each were cut from the flavedo of the proximal, equatorial, and distal areas. The sections were immersed in a 1:10,000 aqueous solution of ruthenium red for 10–30 minutes, cleared in lacto-phenol, and mounted in the clearing solution.

Five stomatal counts were made at random in each section with a microscope outfitted with a 16.2 mm. objective and a 10×ocular. The average number of stomata per microscopic field thus obtained has been reduced to number per square millimeter. Counts were made in 1200 fields.

Oil-gland counts were made on pieces of rind, 8 mm. or more in diameter, cut with a hand razor. Two such surface sections were taken from each of three locations: the proximal, distal, and equatorial areas of the rind of forty fruits picked in a manner similar to that used for the stomatal counts. These surface sections were mounted on glass slides in lacto-phenol clearer and were later transferred to a special counting glass.

The counting glass consisted of two lantern slide covers, 2×2 inches, bound on one edge with cellulose tape to form a hinge. On the outside surface of one of the glasses was cemented a piece of the tape in which a circular hole, 6 mm. in diameter (measured under the microscope), had been cut with a steel spring bow divider.

A section of the rind was placed on the inner surface of this glass directly over the opening in the tape; clearer was added to both surfaces of the rind section; the two glasses were closed together; and the section, thus mounted, was placed in a Leitz VIII S projector equipped with a 250-watt light and an *f*.2.5, 8.5 cm. lens. The image of the circular area of the rind was thrown upon a sheet of paper mounted on a board 263 cm. distant from the lens and showed the oil glands distinctly. Each gland image was numbered on the paper with a pencil, and the final number was recorded. In this manner the oil glands in 240 fields were counted.

Distribution of stomata about oil glands was determined, and an ocular micrometer was used to measure interstomatal and stomatal-oil gland distances. The number of the ring to which a stoma be-

longed with respect to an oil gland as a center and to other neighboring oil glands was also noted. A microscope fitted with an ocular micrometer, a 10×ocular, and a 5.4 mm. objective was used in measuring size of stomata.

## Results

The stomatal apertures of the navel orange fruits averaged 13.0 $\mu$ in length and 5.95 $\mu$ in width. When guard cells were included in the measurements the dimensions averaged 32.4 and 25.5 $\mu$, respectively. Photomicrographs of stomata are shown in figures 1 and 2.

The results of the stomatal counts are recorded in table 1. They show fewer stomata per square millimeter in the proximal area than in the equatorial or distal areas of all fruits studied, and fewer stomata per square millimeter in large oranges than in small ones.

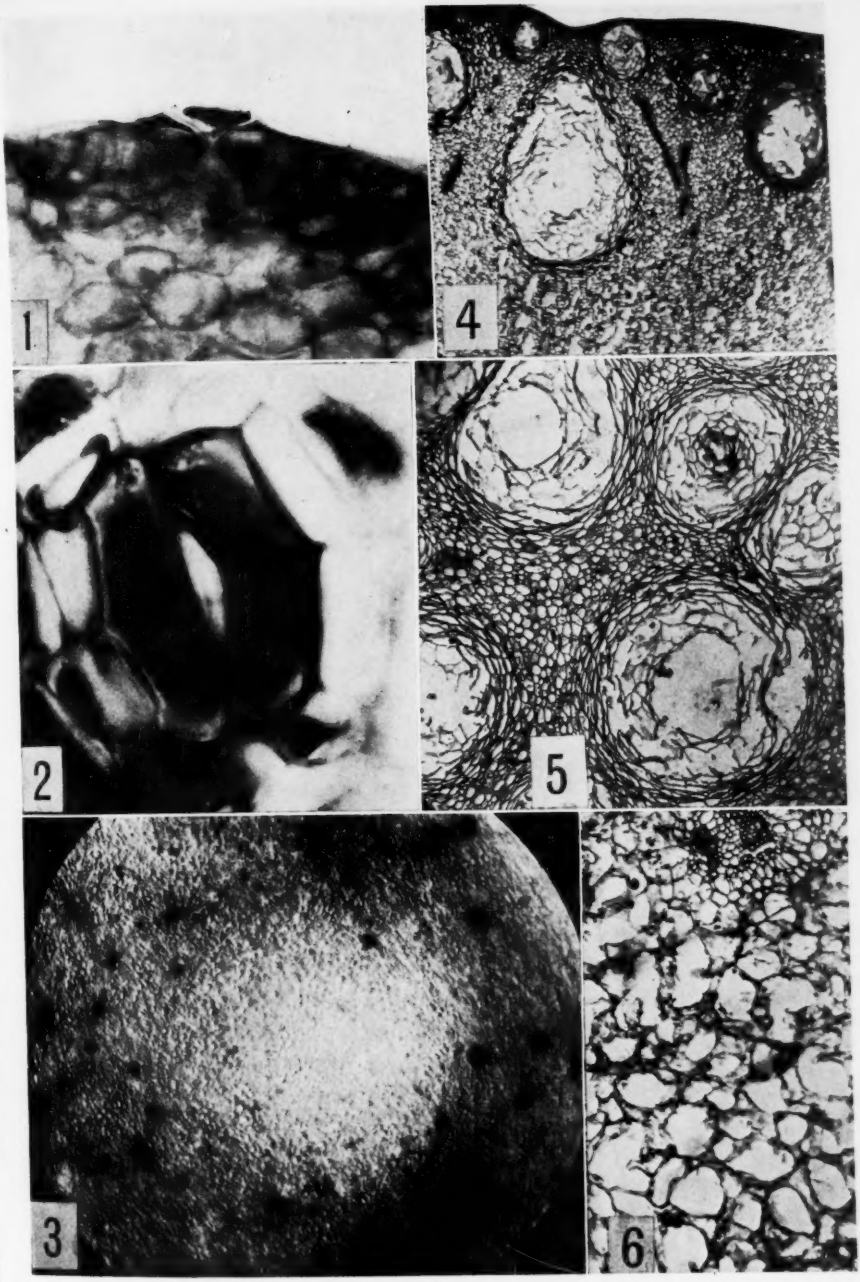
From the mean equatorial diameters and the mean polar diameters of the fruits, the surface area was calculated to be 236.0 cm.$^2$ for the large fruits and 145.4 cm.$^2$ for the small fruits. The equation for the surface of a prolate spheroid (3) is

$$S = 2\pi b^2 + \frac{ab}{e} \sin^{-1} e, \text{ where } e = \frac{\sqrt{a^2 - b^2}}{a}.$$

Further calculation showed that large oranges have on the average a greater total number of stomata (284,600) than small oranges (227,700).

From averages of the oil-gland counts, the mean number of oil glands per square millimeter (table 1) was found to be highest in the proximal area, intermediate in the equatorial area, and lowest in the distal area, when all fruits or large fruits were considered. In small fruits, however, the greatest number of oil glands per square millimeter was found in the proximal area, intermediate number in the distal area, and lowest in the equatorial area. The mean of counts in the proximal, equatorial, and distal areas showed higher concentrations of oil glands in small fruits. Sections through oil glands in the flavedo are shown in figures 4 and 5.

REED and HIRANO (5) noted that oil glands on citrus leaves frequently formed a center about which stomata were distributed. This was observed by us to occur in navel orange fruits, although




FIGS. 1–6.—Rind structures of Washington Navel orange fruit. Fig. 1, radial section showing stoma with guard cells and stomatal plug. Fig. 2, surface view showing stoma with guard cells and stomatal aperture. Fig. 3, small portion of rind showing distribution of stomata around oil gland (light central area); stained with ruthenium red. Fig. 4, radial section showing oil glands. Fig. 5, tangential section showing oil glands. Fig. 6, albedo below oil glands showing cellular network, large intercellular spaces, hesperidin crystals, and vein.

somewhat less definitely than in leaves. It appeared from a study of large-sized fruits that each oil gland was surrounded by one, two, or more rings of stomata, with five to eight stomata in each ring. This circular distribution is shown in figure 7. The distribution about a single oil gland is shown in figure 3. Although wide deviations in the type of distribution were noted, an average of counts

TABLE 1

MEAN DENSITY OF STOMATA AND OF OIL GLANDS PER SQUARE MILLIMETER IN LARGE AND SMALL FRUITS

| Sample | Mean equatorial diameter of fruit (cm.) | Area | | | Mean of proximal, equatorial, and distal areas | Percentage differences of areas* | |
|---|---|---|---|---|---|---|---|
| | | Proximal | Equatorial | Distal | | Proximal and equatorial | Distal and equatorial |
| | Stomata | | | | | | |
| Entire sample | 7.49 | 12.67 | 14.54 | 14.38 | 13.86 | −12.8 | − 1.1 |
| Large fruits | 8.37 | 10.79 | 12.55 | 12.84 | 12.06 | −14.0 | + 2.3 |
| Small fruits | 6.62 | 14.55 | 16.52 | 15.91 | 15.66 | −11.9 | − 3.7 |
| | Oil glands | | | | | | |
| Entire sample | 7.13 | 2.47 | 2.30 | 2.22 | 2.33 | + 7.39 | − 3.48 |
| Large fruits | 8.09 | 2.35 | 2.21 | 1.94 | 2.17 | + 6.34 | −12.21 |
| Small fruits | 6.17 | 2.58 | 2.39 | 2.51 | 2.49 | + 7.95 | + 5.02 |

* Plus or minus signs preceding percentages indicate greater or less density of stomata or oil glands in proximal or distal area than in equatorial area.

of stomata and of measurements between oil gland and stomata of the first ring and of the second ring (rings numbered centrifugally from the oil gland), and an average of distances between stomata in any one ring, showed that on the average a much more uniform distribution occurs than can be judged from observation of isolated cases. An idealized representation, based on average measurements, is shown in figure 8.

In twenty-three samples studied, 0–75 per cent (mean 35 per cent) of the stomata forming the first ring about a given oil gland

were included in the first ring of neighboring oil glands, and 0–88 per cent (mean 25 per cent) were included in the second ring of neighboring oil glands. From 27 to 100 per cent (mean 50 per cent) of the stomata in the second ring about a given oil gland might be included in the first rings of neighboring oil glands, but only 0–37 per cent (mean 14 per cent) were included in second rings. This suggests that oil glands are more regularly surrounded by a single circle of stomata, the secondary rings usually being formed by stomata in the first rings of surrounding oil glands.

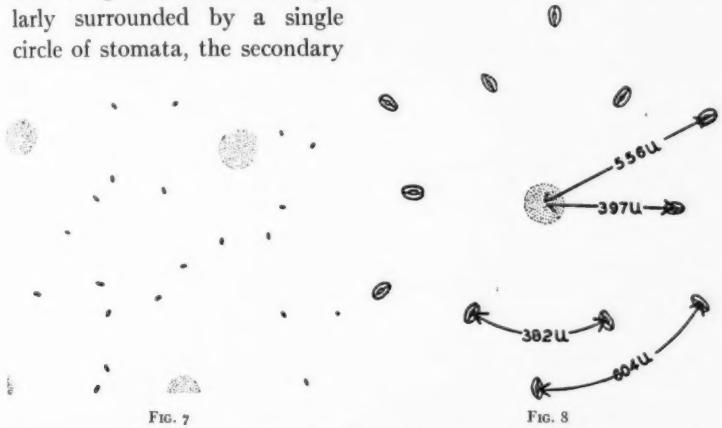



Fig. 7 Fig. 8

Figs. 7, 8.—Fig. 7 (left), semidiagrammatic camera lucida drawing of distribution of stomata about oil glands, 4.3×objective. Fig. 8 (right), idealized representation of distribution of stomata about an oil gland; distances and numbers established as mean of a number of counts.

Examination of both freehand and paraffin sections of untreated flavedo of the Washington Navel orange indicated that numerous stomata were partially or entirely plugged (fig. 1). The plug was generally conical but was frequently observed to be split in the center or unattached to the thick (7.3 $\mu$) cuticle. The chemical nature of the plugging material could not be readily ascertained through microchemical tests.

In order to test the effectiveness of the stomatal plugs, whole untreated oranges were immersed in ruthenium red solution overnight. On removal from the solution it was found that many of the stomata,

and frequently the tissues immediately surrounding them, were colored red, but at no time were all the stomata colored. When portions of an orange were repeatedly treated with benzene and finally wiped with alcohol, many more stomata stained with ruthenium red than when the orange was not so pretreated, and practically all in a treated section stained readily after pretreatment. Apparently the stomatal plug is some type of higher hydrocarbon, soluble in benzene, which is effective in preventing water from rapidly penetrating through a substantial proportion of stomata into the pectinaceous, capillary-like albedo (fig. 6).

In this investigation a lesser stomatal density was found in the Washington Navel orange fruits than was observed by REED and HIRANO (5) in the Washington Navel orange leaf (468 per square mm.), and smaller stomatal sizes were noted than those recorded by them (5) for the Valencia orange leaf ($22.6 \times 17.2\ \mu$). The mean dimensions of the stomatal aperture of the Washington Navel orange fruits have been found to be smaller than the mean aperture dimensions of stomata observed by ECKERSON (1) in leaves of numerous plants ($17.7 \times 6.7\ \mu$), but not so small as the smallest ($5 \times 2\ \mu$) found by her in *Cucurbita pepo*.

A moderately high and highly significant negative correlation exists between stomatal density and equatorial diameter of the fruit, as indicated by the correlation coefficient, $r = -0.597$. The relation is shown by the regression line (fig. 9) fitted by the method of least squares. Density of stomata may readily be determined from the regression line or from the equation of the line, $N = -2.78\,D + 34.75$, $N$ representing the number of stomata per square millimeter and $D$, the equatorial diameter of the orange in centimeters. Such estimations should fall within the standard error of estimate of 4.13 stomata per square mm.

The percentage differences in stomatal and oil-gland density in the proximal area compared with the equatorial area, and in the distal area compared with the equatorial area, are shown in table 2. They indicate that stomatal density is less in the proximal than in the equatorial area, and that it is less in the distal than in the equatorial area, except in large fruits. The difference in the mean stomatal density between the proximal and equatorial areas, when

based on the entire sample, is below the 5 per cent level of significance (table 2) but above the 50 per cent level. Between the means of the distal and equatorial areas the difference in stomatal density is below the 5 per cent level of significance. Based on the mean stomatal density per fruit (average of mean density in proximal, equatorial, and distal areas in each fruit), the difference in the average density in large and small fruits was above the 5 per cent level of significance (table 2).

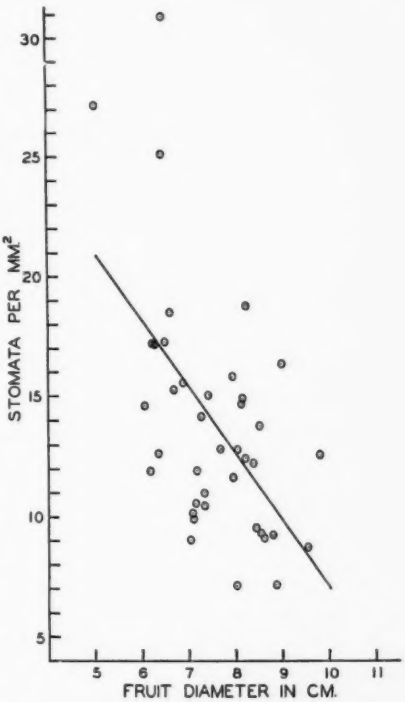



FIG. 9.—Regression line of number of stomata per square millimeter on equatorial diameter of fruit (in cm.).

Oil-gland density is greater in the proximal than in the equatorial area and greater in the distal than in the equatorial area in small fruits, but not in large. Statistical analyses have not been applied to these data; therefore, whether the differences are above the 5 per cent level of significance cannot be stated with surety. The results of the analyses of stomatal density suggest, however, that differences in the mean oil-gland density between different areas on the fruit may also be nonsignificant. Oil-gland density is significantly different in large and small fruits when an average of the mean density in the various areas is used as a basis (table 2). This difference is highly significant since the value of the observed $t$ is above the 1 per cent level of calculated $t$.

Since the incidence of water spot is greatest in the proximal and

distal areas (2), it is evident that there is no positive relation between the concentration of stomata and the incidence of this disorder. Oil-gland concentration, however, suggests a positive correlation with the incidence of water spot in the proximal areas of large and small fruits; but the large negative value (−12.21) with respect to the distal area of large fruits and the small negative value (−3.48) for the distal area of the entire sample indicate no significant correlation with water spot.

Calculation based on data in table 1 showed that small fruits have 29.9 per cent greater stomatal density and 14.24 per cent greater oil-gland density than large fruits. Statistical analyses indicated that these differences were significant. This suggested that the incidence of water spot might be greater in small than in large fruits. When a total of 677 large and small fruits were placed in a rain chamber for 51 hours (91 inches of moisture), however, 47.5 per cent of the small fruits showed water spot in the rind surrounding the navel, as against 50.7 per cent of the large fruits. Similar results were obtained when the navel portions of fifty-two small and forty-one large fruits were immersed in shallow pans of distilled water for 48 hours. As the differences in percentages of injured were not significant, it was concluded that no relation exists between density of stomata or of oil glands and water spot.

TABLE 2

SIGNIFICANCE OF DIFFERENCE OF MEANS OF STOMATAL AND OF OIL-GLAND DENSITY

| COMPARISON | STANDARD DEVIATION OF DIFFERENCE | $t$ OBSERVED |
|---|---|---|
| Stomatal density (all areas), large fruits and small fruits | 1.52 | 2.36 |
| Stomatal density (entire sample), proximal area and equatorial area | 1.30 | 1.44 |
| Stomatal density (entire sample), distal area and equatorial area | 1.30 | 0.12 |
| Oil-gland density (all areas), large fruits and small fruits | 2.92 | 3.27 |

**Summary**

1. The mean stomatal density for Washington Navel orange fruits was 13.86 per square millimeter. Stomatal density was greater in small oranges than in large. The mean density of oil glands was 2.33 per square millimeter.

2. Oil glands were more densely distributed in small oranges than in large. Neither the density of the stomata nor the density of the oil glands is related to the incidence of water spot.

3. The radial arrangement of stomata in narrow circular zones about oil glands, as reported by previous investigators for leaves of citrus, was also found in the rind of the Washington Navel orange fruits.

UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

## LITERATURE CITED

1. ECKERSON, SOPHIA H., The number and size of the stomata. BOT. GAZ. 46:221–224. 1908.
2. FAWCETT, H. S., KLOTZ, L. J., and HAAS, A. R. C., Water spot and water rot of citrus fruits. California Citrograph 18:165, 175. 1933.
3. HODGMAN, C. D., and LANGE, N. A., Handbook of chemistry and physics. Chemical Rubber Publishing Co., Cleveland. 1927.
4. KLOTZ, L. J., Red blotch and peteca of citrus. California Citrograph 15:32, 33. 1929.
5. REED, H. S., and HIRANO, E., The density of stomata in citrus leaves. Jour. Agr. Res. 43:209–222. 1931.

# ORIGIN OF ADVENTITIOUS SHOOTS IN DECAPITATED CRANBERRY SEEDLINGS

HENRY F. BAIN[1]

(WITH FIFTEEN FIGURES)

## Introduction

The hypocotyls of cranberry seedlings (*Vaccinium macrocarpon* Ait.) extend 1–2 cm. above the soil line. If the hypocotyls are severed a few millimeters below the cotyledons soon after emergence of the seedlings, the nodeless stalks almost invariably produce several adventitious buds near their cut ends, one or two of which develop into shoots and carry forward normal growth of the plants (fig. 10). These adventitious shoots originate in the epidermal cell layer.

ROPER (**9**) first reported the occurrence of adventive hypocotyledonary shoots in 1824, describing them on uninjured plants of *Euphorbia exigua*, *E. heterophylla*, and *E. lathyris*. The phenomenon is now known to occur in many species belonging to widely separated families. RAUH (**8**) described and illustrated most of the species known to produce adventive buds and shoots on both hypocotyls and roots, and gives an extensive bibliography. He suggests that these plants can be conveniently classified into three broad groups (examples included in the present review produce hypocotyledonary shoots but not necessarily root shoots) as follows: (*a*) adventive shoots which are obligatory to the development of the plant (Orobanchaceae, Balanophoraceae, Rafflesiaceae, some Loranthaceae, *Linaria supina*, *L. bipartita*, *L. heterophylla*, *L. vulgaris*, *L. genistifolia*, *L. striata*, *Euphorbia amygdaloides*, *Linaria alpina*, and *L. reflexa*); (*b*) adventive shoots which are facultative to the development of the plant, that is, the plant is able to develop to maturity without their formation (annual *Euphorbia* spp., *Antirrhinum majus*, *Linaria spuria*, *L. elatine*, *L. minor*, *Anagallis arvensis*, perennial *Linum* spp., *Alliaria officinalis*, *Isatis tinctoria*, *Anemone silvestris*,

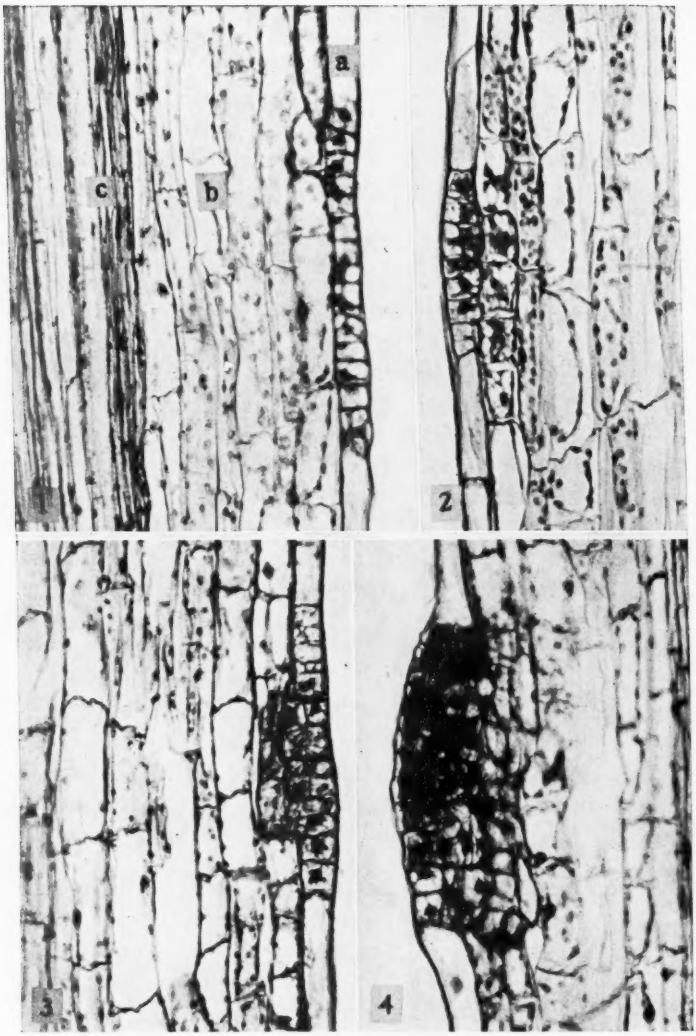
[1] Senior Pathologist; U.S. Horticultural Station, Beltsville, Maryland.

*Geranium sanguineum*, *Paeonia*, *Pulsatilla*, and numerous trees and shrubs); (*c*) adventive shoots which are regenerative, that is the shoots develop only when the seedling is injured or excised below the cotyledons (*Euphorbia bubulina*, *Linaria cymbalaria*, and annual *Linum* spp.). The cranberry belongs in the last group, as do tomato (1) and *Annona muricata* (6). The phenomenon of regenerate bud formation on injured hypocotyls may well be more widespread than is realized at the present time.

Hypocotyledonary buds have been found to originate in the epidermis in the majority of the species in which the mode of origin has been investigated. RAUH (**8,** p. 532) states: "Hypokotylknospen enstehen immer exogen, doch nur an chlorophyllhaltigen, über der Erde befindlichen Hypokotylen, sonst endogen (*Alliaria officinalis*, *Peltaria alliacea*, *Linum flavum*)." He illustrates epidermal bud formation in *Linum usitatissimum*, *L. tenuifolium*, and *Thesium bavarum* (**8,** fig. 83). CROOKS (**4**) and BEALS (**2**) described epidermal bud formation in decapitated flax seedlings (*Linum usitatissimum*). KÜSTER (**5**) states that buds originate exogenously in decapitated seedlings of *Anagallis coerulea* and *Linaria cymbalaria*. BEIJERINCK (**3**) described and illustrated exogenous shoot formation in *Linaria vulgaris*, and VAN TIEGHEM (**10**) confirms exogenous origin in *Linaria* species. PRIESTLEY and SWINGLE (**7,** pl. 10*A*) illustrated epidermal bud origin in *Linaria macedonica*, but state that according to BEIJERINCK (**3**) shoot origin in *Convolvulus arvensis* is endogenous.

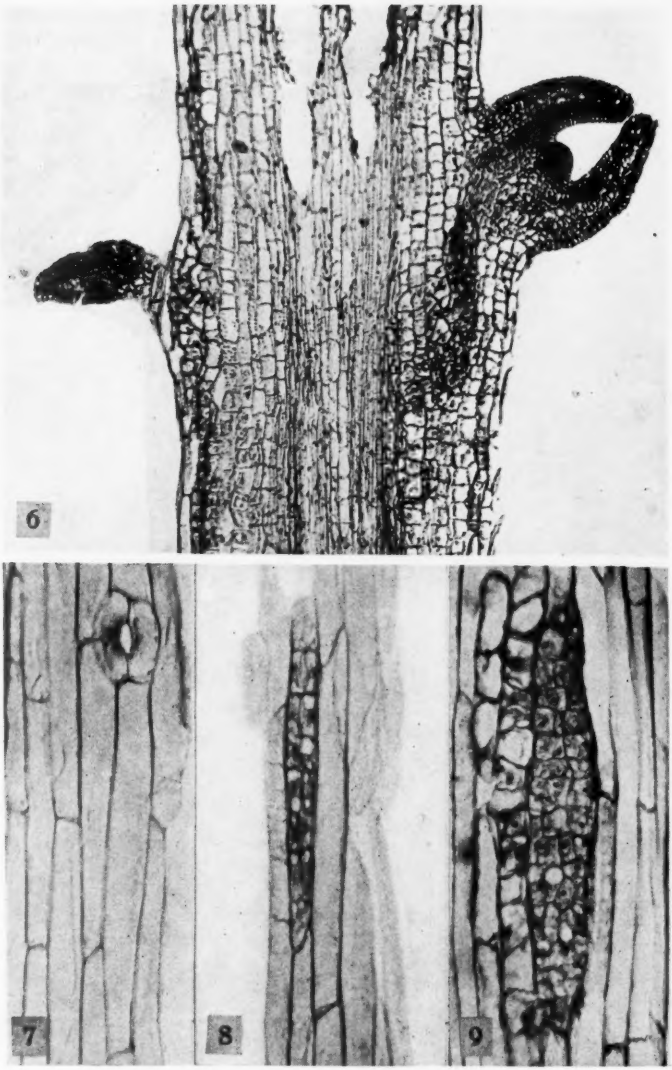
## Material and methods

Cranberry seedlings were grown aseptically in test tubes of water agar. After the cotyledons were fully expanded the plants were decapitated 2–3 mm. below the cotyledons with a bent spear-headed needle. Successive lots of stems were then killed in chromo-acetic acid and in Carnoy's fluid at 4-hour intervals, until buds became visible, and afterward at longer intervals until short shoots bearing leaves were developed. The stems were imbedded in paraffin, cut in sections mostly 8 $\mu$ thick, stained in Heidenhain's haematoxylin, and counterstained in Delafield's haematoxylin. Carnoy's fluid yielded the best results on the whole because of its more rapid penetration of the stems.

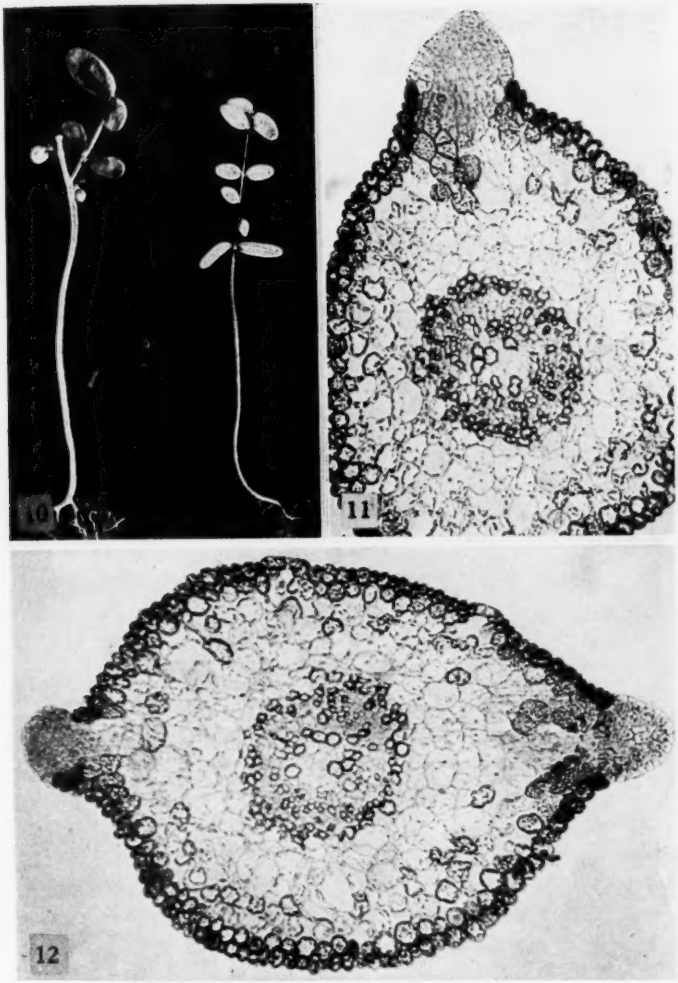



FIGS. 1–4.—Early stages of adventitious bud formation in cranberry seedlings, as seen in median longitudinal sections of stems. Seed planted January 10; decapitated February 12, 1938. Fig. 1*a*, epidermal cell divided by cross walls into several short cells, subepidermal cells scarcely affected; *b*, cortical zone; *c*, central cylinder. Plant killed February 22. Fig. 2, tangential divisions in epidermal cell, subepidermal cells becoming meristematic. Killed February 22. Fig. 3, subepidermal cells dividing. Killed February 23. Fig. 4, cell activity reaching deeper into cortex. Killed February 23.

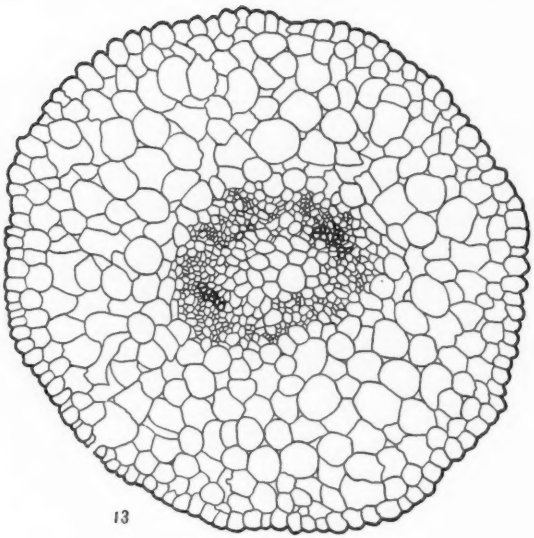
FIG. 5.—Later stage in bud formation, median longitudinal section. Meristematic activity has advanced downward and inward through cortex almost to stem fibrovascular system. Plant killed 11 days after decapitation.



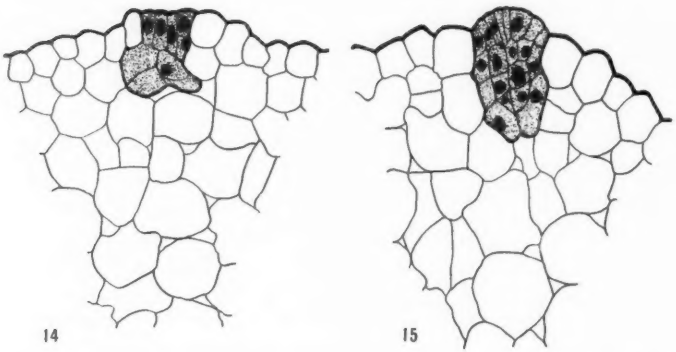

FIGS. 6–9.—Fig. 6, median longitudinal section of stem killed 17 days after decapitation. Fibrovascular system of bud has almost made contact with stem system; second bud on left side of stem is not centrally sectioned. Fig. 7, normal epidermis of hypocotyl as seen in tangential section of stem. Fig. 8, early stage of bud development in tangential section. Within single epidermal cell several cross walls have been laid down, and resulting short cells have undergone one radial division. Plant killed 9 days after decapitation. Fig. 9, later stage showing further divisions in radial plane; adjacent epidermal cells remain unchanged. (Two rows of broad, short cells to left of bud are subepidermal cells exposed because the swelling bud had elevated that portion of epidermis slightly above plane of section.) Stem killed 10 days after decapitation.




FIGS. 10–12.—Fig. 10, seedlings 4 months after planting in water agar. At left, seedling was decapitated below cotyledons and produced five adventitious buds, three of which produced true leaves, although a single shoot finally became dominant. Normal plant at right. Fig. 11, cross section of adventitious bud in upper third of vertical extension; adjoining epidermal cells pushed aside and cortical cells between bud and central cylinder show no signs of meristematic activity. Plant killed 6 days after decapitation. Fig. 12, cross section 80 $\mu$ lower on stem illustrated in fig. 11. Second bud appears at left; bud at right (same bud as in fig. 11) is here cut near bottom of its vertical extension, and at this location meristematic activity, in the form of radial divisions in the cortical cells, can be seen to extend almost to central cylinder.



13



14 15

FIGS. 13–15.—Fig. 13, enlarged drawing from cross section of normal hypocotyl at time plants were decapitated. Fig. 14, early stage of bud development seen in cross section of stem killed 6 days after decapitation. Fig. 15, later stage. Plant killed 6 days after decapitation.

### Hypocotyl

At the time of decapitation the hypocotyl apparently consists entirely of primary tissues. The epidermis is composed of narrow, longitudinally elongated cells and contains numerous stomata (fig. 7). Lying next within the epidermis is a cortical zone about six to ten cells deep, consisting of relatively large, thin-walled, highly vacuolate cells containing numerous chloroplasts (figs. 11, 12, 13). The central cylinder has a diameter somewhat greater than one-third the hypocotyl, and contains probably four poorly defined fibrovascular bundles. The cambial ring has not been completed (fig. 13).

### Adventitious buds

The earliest stage in the development of the buds that could be definitely identified was similar to that shown in figure 1, where a single epidermal cell has been divided by cross-walls into several short cells. Some 2- and 4-celled stages were suspected but in appearance were so similar to the normal irregularly sized epidermal cells that it was uncertain whether the slightly deeper-staining cytoplasmic contents furnished sufficient evidence that active development was beginning. Even the stage reached in figure 1, seen in longitudinal section, could not often be identified in cross sections of the stems.

The meristematic short cells illustrated in figure 1 divide both tangentially and radially (with reference to the stem axis) into extremely small cells (figs. 2, 8). Simultaneously the adjacent cells of the subepidermal layer become active, increasing in size and cytoplasmic content, with the nuclei enlarging (fig. 2). These subepidermal cells soon begin dividing, forming a wedge of meristematic tissue continuous with that initiated in the original single epidermal cell (figs. 3, 4, 14, 15).

Subsequent development takes place outwardly by continued cell division and inwardly toward the central cylinder through meristematic activity of successive cortical cells (figs. 5, 6, 12). The meristematic zone advancing inward to complete connection with the fibrovascular system of the stem follows a downward course through the cortex and eventually makes contact with it some dis-

tance below the developing bud (fig. 6). The available evidence indicates that the outward growth all develops from the tissue originating from a single epidermal cell, adjoining epidermal cells being pushed aside as the bud increases in size (figs. 9, 11, 12).

**Summary**

The cranberry belongs to the increasing list of plants for which hypocotyledonary regeneration of growing points has been demonstrated. Adventive shoots in severed cranberry hypocotyls originate in the epidermal cell layer.

U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. ADAMS, J., Adventitious shoots on hypocotyl of flax and tomato. BOT. GAZ. **78**:461–462. 1924.
2. BEALS, C. M., An histological study of regenerative phenomena in plants. Ann. Missouri Bot. Gard. **10**:369–384. 1923.
3. BEIJERINCK, M. W., Beobachtungen und Betrachtungen über Wurzelknospen und Nebenwurzeln. Verhandl. K. Akad. Wetensch. Amsterdam 25. 146 p. 1887.
4. CROOKS, D. M., Histological and regenerative studies on the flax seedling. BOT. GAZ. **95**:209–239. 1933.
5. KÜSTER, E., Beobachtungen über Regenerationserscheinungen an Pflanzen. Bot. Centbl. Beihefte **14**:316–326. 1903.
6. MENDEL, K., A case of regeneration of the growing point at the hypocotyl. Palestine Jour. Bot. Rehovot Ser. **2**:89–92. 1938.
7. PRIESTLEY, J. H., and SWINGLE, C. F., Vegetative propagation from the standpoint of plant anatomy. U.S. Dept. Agric. Tech. Bull. 151. 1929.
8. RAUH, W., Die bildung von hypocotyl- und wurzelsprossen und ihre bedeutung für die wuchsformen der Pflanzen. Nova Acta Leopoldina N. F. **4**:395–553. 1937.
9. ROPER, J., Enumeratio Euphorbiarum. Göttingen. 1824.
10. VAN TIEGHEM, P., Recherches sur la deposition des radicelles et des burgeons. Ann. Sci. Nat. Bot. (7) **5**:130–151. 1887.

# EFFECT OF NAPHTHALENE ACETIC ACID ON MOBILE AUXIN IN BEAN SEEDLINGS

WILLIAM S. STEWART[1]

(WITH ONE FIGURE)

## Introduction

Application of a 2 per cent lanolin paste of naphthalene acetic acid as a narrow band around the middle of the first internode of 7-day-old kidney bean plants markedly affects their physiology. Some of the treated tissues proliferate rapidly, increasing the stem diameter several times, and there is usually rupturing of the epidermis by many root primordia. In a sufficiently humid atmosphere these primordia develop into roots. The naphthalene acetic acid treatment also causes a mobilization of carbohydrates and nitrogenous materials toward the proliferating tissue. A further effect of the treatment is the inhibition of elongation of the terminal bud (1). It is assumed that these effects are caused by a disturbance in the growth correlation of the plant, and yet no data are available concerning the effect of the treatment on the cell enlargement hormone, auxin, which is known to be related to growth.

WENT and THIMANN (3) restrict the term "auxins," first suggested by KÖGL and HAAGEN-SMIT, to "those substances which bring about the growth reaction which is conveniently measurable by the curvature of *Avena* coleoptiles." As defined, there is no distinction possible between synthetic substances and substances naturally occurring in plants that promote *Avena* coleoptile curvature.

The following investigations were designed to determine the effect of the naphthalene acetic acid treatment on the auxin diffusing from the terminal buds, heart-shaped leaves, and sections of the hypocotyl, and the effect on the auxin diffusing toward these organs from the treated first internode. Auxin collected by diffusion from living organs is spoken of as free-moving or mobile auxin.

[1] Assistant Physiologist, Bureau of Plant Industry, Beltsville, Maryland.

## Experimentation

### Mobile auxin from terminal buds

Determinations of free-moving or mobile auxin have been made in green plants for leaves, stem tissues, and terminal buds (**3**). The method was to place the basal cut surfaces of these parts on small agar blocks. The mobile auxin diffuses into the agar and afterwards is quantitatively measured by the *Avena* test. In this investigation somewhat similar technique was used with 6-day-old kidney bean

TABLE 1

MOBILE AUXIN FROM TERMINAL BUDS OF BEAN SEEDLINGS TREATED WITH 2 PER CENT NAPHTHALENE ACETIC ACID

| EXPERIMENT NO. | AUXIN COLLECTION (HOURS) | NO. OF BUDS PER PLATE | NO. OF AVENA TEST PLANTS PER DETERMINATION | AUXIN FROM TERMINAL BUDS (AS DEGREES AVERAGE NEGATIVE CURVATURE) | |
|---|---|---|---|---|---|
| | | | | UNTREATED | TREATED |
| 52 | First 2 | 12 | 24 | 0 | 10.8±0.9 |
| | Next 5 | | | 0 | 12.6±1.4 |
| | Next 21 | | | 9.1±1.0 | 9.0±0.7 |
| 61 | First 3½ | 16 | 12 | 5.5±0.9 | 16.9±1.7 |
| 62 | First 3½ | 24 | 12 | 0.0± 0 | 11.7±0.7 |
| | Next 23 | | | 10.9±1.5 | 11.0±1.0 |
| 63 | First 5 | 24 | 12 | 3.8±1.3 | 12.2±1.5 |

plants treated with a ring of 2 per cent lanolin paste of naphthalene acetic acid around the middle of the first internode. (The internodes of 6-day-old, soil-grown bean plants are 2–3 cm. long, the hypocotyl about 10 cm. long.)

The effects of the treatment on the mobile auxin of the terminal bud were first investigated. Two hours after application of the lanolin-naphthalene acetic acid treatment the terminal bud was decapitated with a sharp knife, midway of the second internode which at the time was less than 1 mm. long. The terminal bud was placed for 30 minutes with its cut surface on wet filter paper in a 10-cm. petri dish to remove auxin-destroying enzymes resulting from the cutting of the cells (**3**). Twelve buds were then placed on a 1.5 per cent agar plate, 6 × 8 × 1.8 mm. After 2–3 hours they were transferred to blank agar plates where they were left for 4–6 hours, before again being transferred to other plates. They remained on these last for

the duration of the experiment. Experiment no. 52, table 1, was performed in duplicate. The first experiments were made with twelve buds per plate. Later the number was increased to sixteen and then to twenty-four. A corresponding number of buds from untreated plants were used as controls (table 1). In figure 1, the mobile auxin is expressed as the amount diffused into agar per hour. The amount of mobile auxin that diffused from the terminal buds of untreated

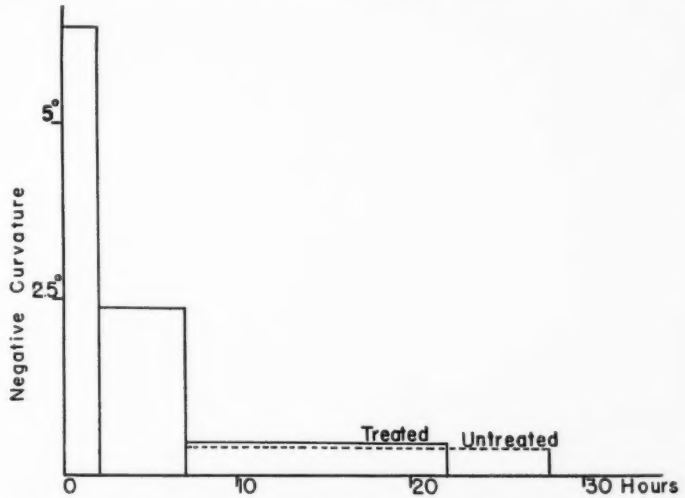



FIG. 1.—Auxin per hour from terminal buds of 6-day-old kidney bean seedlings. Treated by application of 2-mm. wide band of lanolin in 2% naphthalene acetic acid around middle of first internode 2 hours prior to removing buds from plant.

plants was so small that it was detectable only if it was collected for a period of 15 hours. A larger amount of mobile auxin diffused from the terminal buds of treated plants. It is probable that this auxin may be in part naphthalene acetic acid.

## MOBILE AUXIN FROM LEAVES

Mobile auxin from the heart-shaped leaves of 6-day-old bean seedlings was determined by removing them from the plant by a clean cut at the base of the petiole after the 2-hour treatment with naphthalene acetic acid encircling the first internode (the average area per leaf was 11 sq. cm.). The entire leaves were then immersed

for 30 minutes in tap water at the same temperature as the greenhouse where the plants had been growing (24° C.). They were then put between two pieces of water-soaked cellucotton and placed on a stand 1 inch high. The stand was made from $\frac{1}{4}$-inch mesh wire screen. The petioles projected downward about $\frac{1}{2}$ inch through the meshes of the screen. To keep the blades flattened, a piece of glass was put over the top layer of the cellucotton. A 1.5 per cent agar block 2 × 2 × 1.8 mm. was placed on the cut surface of each petiole. The stand was placed in a glass chamber lined with wet filter paper

TABLE 2

MOBILE AUXIN FROM HEART-SHAPED LEAVES OF BEAN SEEDLINGS TREATED WITH 2% NAPTHALENE ACETIC ACID

| EXPERIMENT NO. | AUXIN COLLECTION (MINUTES) | NO. OF LEAVES PER DETERMINATION | NO. OF AVENA PLANTS PER DETERMINATION | AUXIN (AS DEGREES AVERAGE NEGATIVE CURVATURE WITH STANDARD ERROR) | |
|---|---|---|---|---|---|
| | | | | UNTREATED | TREATED |
| 68 | 105 | 30 | 30 | 6.6±0.5 | 10.9±0.9 |
| 70 | 135 | 21 | 21 | 9.1±1.0 | 11.3±1.0 |
| 109 | 180 | 50 | 50 | 12.2±0.5 | 11.6±0.5 |
| 110 | 105 | 40 | 40 | 10.1±0.5 | 8.1±0.4 |

and kept in a dark control room at 24.5° C. By this technique mobile auxin from the petiole was collected in the agar block and determined quantitatively by the *Avena* test (table 2). The amount of auxin which diffused from leaves of treated plants was not significantly different from the amount diffusing from leaves of untreated plants.

## MOBILE AUXIN FROM HYPOCOTYL

The amount of auxin which diffused from sections of the hypocotyl from treated plants was compared with the amount from untreated plants. Sections 1 cm. long were cut from the middle and base of the hypocotyl. They were placed upright on moist filter paper as described for the terminal buds and then transferred to 1.5 per cent agar plates 6 × 8 × 1.8 mm. in size. Another agar plate of the same size was placed on the upper surfaces. Five sections per plate were used. In experiment 72B after $2\frac{1}{2}$ hours, and in experiment 74 after $4\frac{1}{2}$ hours, the plates were cut into twelve blocks of equal size

and analyzed for auxin. There was more auxin from the mid-hypocotyl sections of treated plants than from untreated ones (table 3), but no significant difference for the basal sections.

Whether the increased amount of auxin was owing to increased production of a naturally occurring mobile auxin, perhaps from some precursor, or whether it was auxin coming from the externally applied naphthalene acetic acid was a matter of question.

TABLE 3

MOBILE AUXIN FROM PIECES OF HYPOCOTYL OF BEAN SEEDLINGS TREATED 2 HOURS WITH 2 PER CENT NAPHTHALENE ACETIC ACID

| Experiment no. | Auxin collection from hypocotyl | Auxin (degrees average negative curvature of twelve Avena test plants) | |
|---|---|---|---|
| | | Untreated | Treated |
| 74 | Middle section Upper surface | 0 ± 0 | 7.6±0.8 |
| 74 | | 2.3±1.0 | 7.1±1.6 |
| 72B | | 3.6±1.0 | 12.1±1.1 |
| 74 | Middle section Lower surface | 6.5±1.4 | 9.3±0.5 |
| 74 | | 1.8±0.9 | 6.5±1.6 |
| 72B | | 5.4±0.8 | 17.3±0.8 |
| 74 | Basal section Upper surface | 0 ± 0 | 0 ± 0 |
| 74 | | ..... | 0 ± 0 |
| 72B | | 4.7±1.1 | 1.5±0.8 |
| 74 | Basal section Lower surface | 1.6±0.8 | 6.8±0.7 |
| 74 | | 5.4±0.7 | 7.1±0.7 |
| 72B | | 0 ± 0 | 3.6±1.2 |

## MOBILE AUXIN DIFFUSING TOWARD TERMINAL BUD, LEAVES, AND HYPOCOTYL

Since the naphthalene acetic acid treatment caused an increase in the mobile auxin diffusing out of the terminal buds and hypocotyl sections removed from the plant, the question was raised as to whether or not this auxin came from the first internode, the site of application of the naphthalene acetic acid. Accordingly the mobile auxin diffusing away from the first internode and toward these organs was measured.

Six-day-old kidney bean plants were grown in soil as in the first experiments. They were then selected for uniformity of size and

treated at the middle of the first internode with 2 per cent lanolin paste of naphthalene acetic acid as before. Immediately after treatment a 3 per cent agar block (2 × 2 × 1.8 mm.) was put at one of the following places on the plant: (*a*) on the stump of the second internode after decapitation of the terminal bud; (*b*) on the stump (about 1 mm. long) of the petiole after removal of one of the heart-shaped leaves; (*c*) on the cut end of the midrib of the leaf blade about 2 mm. beyond the junction of the petiole and blade;[2] (*d*) on the remaining stump of one of the cotyledons after its removal; and (*e*) on the lower half of the hypocotyl in a small incision about 1 mm. deep. The incisions into the leaf and hypocotyl were made under water to prevent air from entering the vessels. Twelve plants were used for each location of the agar blocks. The experiment was repeated for the petiole stump, leaf blade, and hypocotyl. In all cases as soon as the agar block was in position the whole plant was covered with a glass jar lined with moist paper. During days of bright sunlight the plants were shaded. The agar blocks were allowed to stay on the plants for 2 hours, the same length of time allowed for treatment in the first series of experiments. They were then tested for auxin. Generally agar blocks from comparable positions on treated and untreated plants had dried out to the same extent, so that direct comparisons of the auxin could be made. The size of the block usually did not decrease more than half. The greatest drying occurred with agar blocks applied to the hypocotyl (table 4).

An effect of the naphthalene acetic acid treatment is to cause an increase in the mobile auxin from the stump of the second internode, petiole, and hypocotyl.

It is possible then that the observed increase in mobile auxin from the terminal bud and hypocotyl was caused by an increased amount of mobile auxin resulting from the treatment of the first internode. These data suggest that since the auxin was moving acropetally, against the usual polar transport for auxin, it was possibly being carried in the transpiration stream. This effect of the transpiration stream on auxin transport has been observed with cuttings where

[2] The leaf blade was left intact—except for a narrow strip cut out along each side of the midrib and including it, from the place where the agar block was placed to the tip of the blade.

auxin at high concentrations in water solutions was applied at basal cut surfaces (3).

Experiments to determine the effect in 2 hours of naphthalene acetic acid treatment on the mobile auxin in the roots were performed by allowing the main tap root of bean seedlings to grow through the opening in the bottom of 4-inch flower pots. Seventy-two 7-day-old seedlings, grown in soil, were watered 2 hours before

TABLE 4

AUXIN IN BEAN SEEDLINGS DIFFUSING AWAY FROM SITE OF 2 PER CENT NAPHTHALENE ACETIC ACID TREATMENT IN 2 HOURS

| EXPERIMENT NO. | LOCATION OF AUXIN COLLECTION | AUXIN (AS DEGREES AVERAGE NEGATIVE CURVATURE OF TWELVE AVENA COLEOPTILES) | |
|---|---|---|---|
| | | UNTREATED | TREATED |
| 69 | Stump of second internode | 0 ± 0 | 7.5±1.0 |
| 71A | Stump of petiole | 0 ± 0 | 5.5±2.6 |
| 73 | Stump of petiole | 0 ± 0 | 6.3±1.4 |
| | Incision in leaf blade | 1.8±0.9 | 8.2±0.9 |
| | Stump of cotyledon | 4.2±1.6 | 6.0±1.6 |
| | Incision in hypocotyl | 1.5±1.4 | 5.1±1.2 |
| 77 | Incision in leaf blade | 5.8±1.0 | 15.5±1.3 |
| | Incision in hypocotyl | 0 ± 0 | 4.1±2.1 |

the experiment began. Half of them had the band of 2 per cent lanolin-naphthalene acetic acid around the first internode as usual. The root was cut off 1 cm. below the pots and a 3 per cent agar block, 2 × 2 × 1.8 mm., applied to the cut surface. The pot was then set over a jar containing water. Two hours later the agar blocks were removed and analyzed for auxin. No auxin was found in the blocks from the roots of the treated or untreated plants.

## ETHER-EXTRACTABLE AUXIN

As already shown, the naphthalene acetic acid treatment increased the mobile auxin in bean seedlings. The effect of this increase on the ether-extractable growth substances was next investigated. The

roots, hypocotyl, cotyledons, first internode, heart-shaped leaves, and terminal bud were each extracted separately 6, 20, 44, and 96 hours after the usual naphthalene acetic acid treatment. Extractions were made from the corresponding parts of untreated plants at the same times. Extractions of untreated plants were also made at the beginning of the experiment. The lanolin-naphthalene acetic acid paste was not included in the internode extractions, as the treated portion of the internode, about 4 mm. long, was cut out and only the remaining parts which had no paste on them were extracted.

The results are difficult of interpretation owing to the fact that a substance was extracted along with the auxin which caused a growth inhibition of *Avena* coleoptiles, thus rendering the standard *Avena* test for auxin inadequate, as pointed out in a previous paper (**2**). Positive curvatures of 25°, in $2\frac{1}{2}$ hours after the beginning of the *Avena* test, were obtained with ether extracts of the leaves. The greatest amounts of inhibitor extracted were from the heart-shaped leaves and first internodes of 8-day-old seedlings. In the same plants the greatest amount of auxin was extracted from the terminal bud.

Extractions of the first internode of untreated and treated plants 6 hours after treatment showed respectively, 90 minutes after application of the agar blocks to the *Avena* coleoptiles, negative curvatures of 0° and 36° (calculated on 100 gm. fresh weight of material extracted; extract taken up in 0.3 cc. of 1.5 per cent agar). Sixty minutes later positive curvatures of 29° and 9°, respectively, were found. This apparent decrease in the inhibitor content of the treated internodes, as shown by the *Avena* coleoptile curvatures, was possibly caused by naphthalene acetic acid extracted from the internode masking the inhibitor present.

## Discussion

In untreated kidney bean seedlings the terminal bud is the site of the highest concentration of ether-extractable auxin but not of free-moving or mobile auxin (tables 1 and 2). Because the terminal bud is actively growing there appears to be a translocation of food materials in its direction. In *Avena* coleoptiles the tip is likewise the site of the highest concentration of ether-extractable auxin; but in contrast to the terminal bud of the bean seedling, it also produces the greatest amount of mobile auxin (**3**). In 6-day-old bean plants the

greatest amount of mobile auxin is produced by the heart-shaped leaves. An ether extraction of these leaves shows not only the presence of auxin but also a substance which causes positive *Avena* coleoptile curvatures. A similar situation with radish has been described (2).

In treated bean seedlings, in contrast to untreated, the first internode is a site of high concentration of both mobile and ether-extractable auxin (table 4). Whether this is mainly naphthalene acetic acid or whether it is a naturally occurring auxin has not been determined. Thus, in less than 2 hours, the naphthalene acetic acid treatment results not only in a new gradient of mobile auxin (away from the first internode), but also increases the amount of mobile auxin in the parts of the plant studied (tables 1, 3, and 4). The presence of a high content of mobile and bound auxin near the treated portion is associated with a mobilization of carbohydrate and nitrogenous materials toward that portion, and not toward the terminal bud as in the untreated plant (1).

## Summary

In 7-day-old bean seedlings, applications of a 2 per cent lanolin paste of naphthalene acetic acid applied as a band 1–2 mm. wide around the middle of the first internode caused in less than 2 hours an increase in the amount of mobile auxin in the terminal bud, internode, and hypocotyl. The treatment likewise established a new gradient of mobile auxin. It also increased the ether-extractable auxin (which includes the mobile auxin) in the first internode. Ether extractions of bean seedlings showed large amounts of auxin in the terminal bud, while in other parts of the plant a substance was found capable of causing *Avena* coleoptile growth inhibition.

U.S. Horticultural Station
Beltsville, Maryland

## LITERATURE CITED

1. Mitchell, J. W., and Stewart, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. Bot. Gaz. 101:410–427. 1939.
2. Stewart, W. S., A plant growth inhibitor and plant growth inhibition. Bot. Gaz. 101:91–108. 1939.
3. Went, F. W., and Thimann, K. V., Phytohormones. Macmillan, New York. 1937.

# GROWTH OF INCOMPATIBLE POLLEN TUBES IN OENOTHERA ORGANENSIS

STERLING EMERSON

(WITH SIX FIGURES)

## Introduction

Self-sterility is rather widely distributed among the flowering plants, and in certain instances (see **2** and **7** for references) the cytological and physiological aspects of the phenomenon have been investigated, but in no case is the underlying cause of self-sterility understood. Different plants have given rather diverse results, and *Oenothera organensis* appears to differ in several respects from other organisms so far studied. Numerous negative data relating to the physiology of self-sterility in this species have been accumulating during the last few years, which, while giving no direct lead to an attack on the problem, will be discussed briefly in part I of this paper in order to show something of the limits of the problem and to make more understandable the experiments reported here.

## I. Self-sterility in Oenothera organensis

### Genetic basis of self-sterility

The way in which self-sterility is inherited in *Oenothera organensis* is identical with that originally worked out by East and Mangelsdorf (**4**) in *Nicotiana*. This so-called oppositional-factor relationship is apparently the most common basis for self-sterility in the flowering plants (**7**). In *O. organensis* there is a series of forty-five allelomorphs which governs the self-sterility response (**5, 6,** and unpublished). Two members of this series, which is commonly designated by the symbols $S_1$, $S_2$, $S_3$, . . . . $S_n$, are present in the diploid tissues of every individual, the haploid pollen and embryo sacs having one or the other in equal proportions. Incompatibility is a gametophytic character, in that the response of the pollen is determined by the particular allelomorph carried by it, with no influence from the second allelomorph present in the parental sporophyte. Pollen carrying any particular allelomorph, say $S_1$, fails to produce normally

developing pollen tubes in any style in which the same allelomorph is present, $S_1/S_2$, $S_1/S_3$, . . . . $S_1/S_n$, but does produce functioning pollen tubes in any style not carrying that particular allelomorph, $S_2/S_3$, $S_7/S_{26}$, etc. Hence crosses between different individuals may behave in any of three distinct ways: (1) in crosses between plants of identical constitutions, such as $S_1/S_2 \times S_1/S_2$, neither $S_1$ nor $S_2$ pollen can grow in the $S_1/S_2$ style and the combination is completely cross-sterile; (2) in crosses between plants having one allelomorph in common, as in $S_1/S_2 \times S_1/S_3$, pollen carrying $S_1$ fails to function in the $S_1/S_2$ style but $S_3$ pollen develops normally, its nuclei eventually combining with the two sorts of eggs to give seeds of the constitution $S_1/S_3$, identical with the pollen parent, and $S_2/S_3$, which is unlike either parent; (3) in crosses such as $S_1/S_2 \times S_3/S_4$, in which the parents have no allelomorph in common, both $S_3$ and $S_4$ pollens function to give seeds of four different constitutions—$S_1/S_3$, $S_2/S_3$, $S_1/S_4$, and $S_2/S_4$, none of which is identical with either parent. This scheme of inheritance has been established for *O. organensis* both by progeny tests and by direct observation of pollen-tube behavior within the tissues of the styles (**5**).

### Pollen-tube growth in stigma and style

On the stigma, both compatible and incompatible pollens (having respectively neither or one of the allelomorphs present in the pistillate parent) germinate within 15 minutes. At first there is no definitely distinguishable difference between the two sorts of pollen tubes, but later, generally within the first hour after pollination, the incompatible tubes cease growing and remain short while the compatible tubes continue to grow down through the style to the ovary.

As the compatible pollen tubes grow down the style, the entire cell contents pass down the tubes, leaving the pollen grains empty and shrunken. Incompatible pollen tubes ordinarily do not grow sufficiently long to empty their cell contents. This distinction between compatible and incompatible pollen becomes evident within 3 hours after pollination. Photomicrographs of this condition have been published (**5**, figs. 3–6).

The growth rate of compatible pollen tubes is constant (**5**) and at ordinary temperatures is in the neighborhood of 6–8 mm. per

hour. Compatible pollen tubes generally reach the ovary, a distance of 150–180 mm. in most flowers,[1] within 24 hours after pollination.

The maximum growth attained by incompatible pollen tubes may vary (*a*) with the season, (*b*) among different flowers from a single plant, (*c*) among different plants of the same constitution, and (*d*) according to the allelomorph present. Seasonal variation will be discussed later, in the section on "end-season fertility." Examples of variation among flowers from the same plant can be found in the controls in tables 4 and 5. Among plants carrying the same self-sterility allelomorphs some may show much more variability than others. These differences are perhaps due to genetic dissimilarities not associated with the self-sterility allelomorphs, and the effects may be very indirect. For the most part, variation in the response of incompatible pollen tubes is not sufficiently great to obscure the distinction between compatible and incompatible tubes if they have been allowed to grow 4 hours or longer. The chief exceptions to this condition are the responses of self-sterility allelomorphs $S_9$ and $S_{39}$.

In the seasons of 1937 and 1938, considerable difficulty was experienced in identifying allelomorph $S_9$. In many styles carrying this allelomorph, pollen tubes also carrying $S_9$ grew long enough to empty the pollen grains of their cell contents and make them indistinguishable from compatible pollen grains. In other styles from the same plants, however, pollen carrying $S_9$ gave the normal incompatible response. In the current season (1939) this difficulty largely disappeared, perhaps owing to changed environmental conditions—but equally likely to an increased familiarity with the material on my part, which enabled a clearer distinction to be made between the response of allelomorph $S_9$ and compatible allelomorphs.

The incompatible response of allelomorph $S_{39}$ is still more extreme than that of $S_9$. At the height of the flowering season it is common for at least part of the tubes carrying $S_{39}$ to grow as rapidly in incompatible styles as in compatible styles for at least 10 hours after pollination. Direct examinations of pollen-tube growth within the

[1] Growth of pollen tubes is not measurably different in flowers removed from the plant from that in flowers left attached. Unless otherwise noted, the procedure followed was to remove the flowers before pollination and store them in moist chambers during the interval in which the pollen tubes were allowed to grow.

styles were not made after longer intervals, but whether or not the pollen tubes reached the ovaries, no seeds set following self-pollination, indicating that $S_{39}$ is also a sterile allelomorph. Flowers were heavily pollinated (500–1000 pollen grains) about 8 hours before normal anthesis and again 24 hours later, the second pollination coinciding with the time at which pollinations are made for the usual tests. No seeds were obtained from twenty-seven such pollinations,

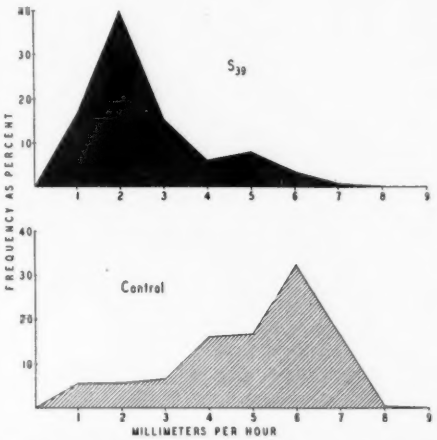



FIG. 1.—Frequency distribution of pollen tubes having different growth rates. Upper curve represents growth of pollen carrying $S_{39}$ in incompatible styles (based on measurements of 1888 pollen tubes made 3–10 hours after pollination, in 25 different styles). Lower curve represents growth of compatible pollen tubes in styles of same plants (measurements of 641 tubes in 22 flowers at equivalent times).

whereas every flower pollinated in the same manner—but with compatible pollen—has given a good set of seeds during the current season. When flowers heterozygous for $S_{39}$ are self-pollinated, nearly all pollen tubes carrying that allelomorph grow sufficiently long to empty the pollen grains of their contents, giving an appearance exactly similar to that found when a pollination is made between two flowers having one allelomorph in common.[2] But comparatively

[2] The plant used in this instance was $S_7/S_{39}$. Crosses using this plant as pollen parent showed that $S_7$ gave the typical short tube, incompatible response in all styles also carrying $S_7$; similarly, pollen carrying $S_7$ from other sources always gave the typical short-tube response in styles of $S_7/S_{39}$. The atypical response of half the pollen tubes in styles of $S_7/S_{39}$ following self-pollinations must therefore be attributed to $S_{39}$.

few of the tubes carrying $S_{39}$ grow as rapidly as compatible tubes, giving a different distribution of tubes within the style (fig. 1).

In certain self-sterile plants (for example in *Abutilon;* **7,** pl. I, fig. 2) incompatible pollen tubes become swollen at the tips, or burst. This condition does not exist regularly in *Oenothera organensis*. In occasional flowers pollen tubes with swollen tips have been observed, but these have nearly always been found following completely compatible pollinations. This condition in *O. organensis* must be attributed to some peculiarity of the style of the particular flowers in which it occurs, since the pollen has reacted normally in other styles. Swollen pollen tubes have been observed more frequently in flowers from plants grown in the greenhouse during the winter than from field-grown plants.

Three-quarters of an hour after pollination, when compatible and incompatible pollen tubes are of similar lengths and near the maximum length of incompatible tubes, there is no striking difference between them. In some compatible tubes the starch grains appear to be digested to a greater extent at this time, but there is considerable variation from one tube to another and the difference has not been established.

### Bud fertility and end-season fertility

In many self-sterile plants (such as *Nicotiana,* **1**) seeds may be obtained following self-pollinations provided either (*a*) the stigmas are pollinated long before the flowers would normally open (bud fertility), or (*b*) the flowers have been produced at a time when the plant has nearly stopped flowering (end-season fertility). Neither of these types of so-called pseudo-fertility occurs in *O. organensis*. The stigmas of this species are not receptive to pollen much earlier than 36 hours before normal anthesis. If they are pollinated earlier the pollen fails to germinate, and whenever the stigma is receptive both compatible and incompatible pollens respond as on open flowers. When plants are at the end of their flowering period the tubes from incompatible pollen often grow longer than at the height of the flowering season, but no seed has ever been obtained following self-pollination under these conditions. *O. organensis* has proved to be one of the most completely self-sterile species so far investigated. None of the methods by which seeds from self-pollinations may be obtained in other species is effective in this one.

### Localized inhibitory zones

East (3) suggested, on the basis of growth rates of compatible and incompatible pollen tubes in immature styles—as contrasted with that of compatible pollen tubes in mature styles—that there is a localized zone near the base of the stigma in *Nicotiana* within which inhibition of incompatible pollen tubes takes place. Sears (7) found that the block to the growth of incompatible pollen tubes in *Brassica oleracea* var. *italica* was at the surface of the stigma. If the surface was removed, incompatible tubes grew nearly as well as compatible, giving nearly as good a set of seeds.

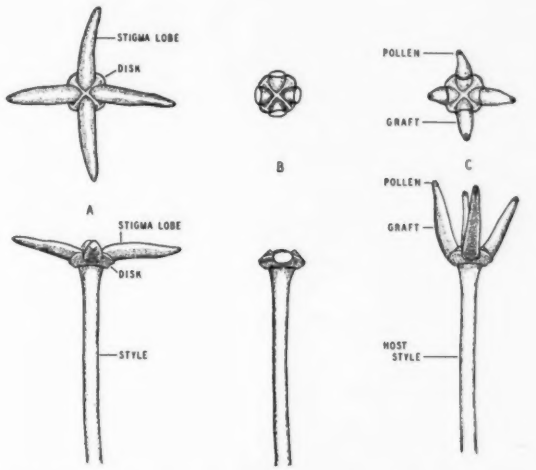



Fig. 2.—Method of making stigma grafts. Compatible pollen is placed on tips of grafted stigma lobes and tube growth measured below graft in either compatible or incompatible tissue, as the case may be.

No such localized block seems to be present in *Oenothera organensis*. Whenever accurate measurements of pollen-tube length are to be made, it is customary to cut off the four lobes of the stigma (as in *B*, fig. 2) and pollinate the more-or-less hemispherical surface remaining, so that all tubes have the same distance to grow before entering the style. Incompatible pollen tubes grow no longer from the cut surfaces than from the intact surfaces of such stigmas. The results of certain style and stigma grafts bear on this point.

Stigma grafts were made in the manner illustrated in figure 2. The four lobes of the stigma of one constitution, say $S_1 / S_2$, were cut off and the stigma lobes of a different constitution, $S_3 / S_4$,

TABLE 1

GROWTH OF POLLEN TUBES IN COMPATIBLE AND INCOMPATIBLE TISSUES AFTER PASSING THROUGH COMPATIBLE STIGMA GRAFTS (FIG. 2)

| STYLE | GRAFT | POLLEN | HOURS AFTER POLLINATION | LENGTHS OF POLLEN TUBES BELOW GRAFT (MM.) | | | | | | | | | | | MEAN LENGTH PER HOUR (MM.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.1–10 | 10–20 | 20–30 | 30–40 | 40–50 | 50–60 | 60–70 | 70–80 | 80–90 | 90–100 | 100–110 | 110–120 | |
| $S_1/S_2$.... | $S_3/S_4$ | $S_1/S_2$ | 8 | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.2 |
| | | | 10 | 3 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.9 |
| | | | 20 | 3 | 2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.4 |
| $S_3/S_4$.... | $S_3/S_4$ | $S_1/S_2$ | 8 | .... | .... | .... | 4 | .... | .... | .... | .... | .... | .... | .... | .... | 4.4 |
| | | | 20 | 1 | ... | ... | ... | ... | ... | ... | ... | 3 | ... | 1 | 1 | 3.9 |
| $S_3/S_4$.... | $S_1/S_2$ | $S_3/S_4$ | 8 | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.2 |
| | | | | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.1 |
| | | | 10 | ... | ... | 1 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 3.0 |
| | | | | 1 | 1 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1.2 |
| | | | 20 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.2 |
| $S_1/S_2$.... | $S_1/S_2$ | $S_3/S_4$ | 8 | ... | 1 | 3 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | 3.5 |
| | | | | ... | ... | ... | 1 | 2 | 1 | ... | ... | ... | ... | ... | ... | 6.1 |
| | | | 10 | 2 | ... | ... | 3 | 5 | 3 | ... | ... | ... | ... | ... | ... | 4.5 |
| | | | | ... | 1 | 1 | 2 | ... | ... | 1 | ... | ... | ... | ... | ... | 3.7 |
| | | | 20 | 3 | ... | ... | 2 | 1 | 3 | 1 | ... | ... | ... | ... | ... | 1.7 |
| | | | | ... | ... | ... | 1 | 1 | 1 | 2 | 3 | ... | 2 | ... | ... | 3.4 |
| $S_1/S_2$.... | None | $S_1/S_2$ | 8 | 50 | 7 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.3 |
| | | | 10 | 47 | 14 | 5 | 2 | 1 | ... | ... | ... | ... | ... | ... | ... | 0.9 |
| | | | 20 | 55 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.3 |
| $S_3/S_4$.... | None | $S_3/S_4$ | 8 | 62 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.4 |
| | | | 10 | 54 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.5 |
| | | | 20 | 32 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 0.3 |

were grafted on in their places. If the cuts are cleanly made with a sharp blade the stigma lobes to be grafted on adhere strongly when the cut surfaces are brought in contact and no adhesive has been used. The grafted stigma lobes were pollinated at their tips with pollen of the same constitution as the host stigma, $S_1 / S_2$ in this

instance, which is compatible with the tissues above the graft union and incompatible with those below. As a control, stigma lobes $S_3 / S_4$ were grafted on to host stigmas of the same constitution and again pollinated by $S_1 / S_2$, which is compatible with the tissues below the graft union as well as above. The data from one such experiment are summarized in table 1.

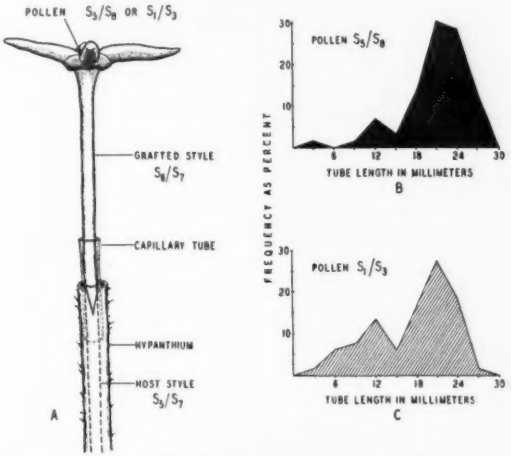



FIG. 3.—Pollen-tube growth in grafted styles. *A*, method of making grafts; *B*, distribution of pollen tubes in compatible grafted styles when host style is incompatible with one of allelomorphs carried in pollen; *C*, distribution of pollen tubes in compatible grafted styles when host style is compatible with both allelomorphs.

Six of the eight test grafts gave approximately as little tube growth below the graft unions as the incompatible controls, whereas each of the eight control grafts (compatible) showed much greater growth. In two of the test grafts the incompatible tubes grew at nearly the rate of compatible tubes in the control grafts, but in other experiments with the same plants incompatible pollinations sometimes gave as much growth, and it is thought that there was no effect of grafting in this experiment.

Grafts of a similar sort were made at varying distances down the style. These were made essentially as illustrated in figure 3*A*, but the grafts were made after the stigmas had been pollinated, at a time when the longest tubes were nearly at the level to be cut. In

these grafts comparatively few pollen tubes cross the graft unions, but the few data obtained were consistent with the interpretation that the incompatibility response takes place at any level in the stigma or style.

In another experiment stigma lobes of one constitution, $S_1 / S_2$, were grafted directly on to the upper surfaces of ovaries of another constitution, $S_3 / S_4$, and pollinated with pollen ($S_3 / S_4$) which was compatible with the grafted stigma lobes but incompatible with the ovaries. Although many attempts were made, no seed was obtained from pollinations of this sort, indicating that the incompatibility response may occur in the ovary also.

These experiments indicate that the incompatibility response is not confined to any particular zone of the style or stigma in *O. organensis*. This conclusion is also borne out by the behavior of pollen tubes carrying allelomorph $S_{39}$, already referred to, and by the behavior of incompatible pollen tubes in styles from plants kept in the dark, to be discussed in the second part of this paper, in both of which the response is so weakened that tubes grow to considerable lengths before their growth is stopped.

These experiments also support the conclusion, previously stated by EAST and others, that incompatible pollen tubes are inhibited in some way upon coming in contact with the tissues of the stigma or style, rather than that compatible pollen tubes receive some necessary growth stimulus from compatible tissues. If the latter alternative were true, pollen tubes in the experiments just described should continue rapid growth in the incompatible tissues below the graft unions as long as cytoplasm remained in the portions of the tubes above the graft unions where they were in direct contact with compatible tissues. This is not the case.

## DIFFUSIBLE SUBSTANCES

From experiments with *Petunia violacea*, YASUDA (**8, 9, 10**) concluded that substances diffusing from the placental region of the ovary controlled the growth of compatible and incompatible pollen tubes within the styles. He made various style grafts and found that the response of the pollen tubes was always that to be expected from the genetic constitution of the ovaries, regardless of the constitution of the stylar tissues through which the tubes were growing.

Pollen tubes grown in vitro showed the same response when pieces of placental tissues were added to the solutions.

Similar style grafts were made with *O. organensis*. In my material, however, the response of pollen tubes always depended upon the constitution of the tissues through which they were growing, with no noticeable effect from the ovary. Grafts were made as indicated in figure 3*A*. The hypanthium and style were cut about 50 mm. below the stigma. The cut ends of the host style and of the

TABLE 2

GROWTH OF POLLEN TUBES IN GRAFTED STYLES. ALL GRAFTS WERE $S_6/S_7$ ON TO $S_5/S_7$ STYLES (FIG. 3). THE PLUS SIGN INDICATES A COMPLETELY COMPATIBLE COMBINATION; THE FRACTION $\frac{1}{2}$, A COMBINATION IN WHICH HALF THE POLLEN IS COMPATIBLE AND HALF INCOMPATIBLE

| Pollen | Reaction | | Frequencies of Tubes | |
|---|---|---|---|---|
| | Graft | Host | 0.1–15 mm. | 15.1–30 mm. |
| $S_1/S_3$ | + | + | 12 | 16 |
| | | | 11 | 26 |
| $S_5/S_8$ | + | $\frac{1}{2}$ | 8 | 51 |
| | | | 7 | 47 |
| | | | 6 | 30 |
| | | | 5 | 48 |
| | | | 3 | 32 |
| | | | 8 | 40 |

style to be grafted on to it were brought in contact inside a capillary glass tube of suitable diameter, which was wedged into the hypanthium for support. The whole flower, still attached to the plant, was covered with a small moist chamber and allowed to stand overnight. Grafted stigmas which showed no signs of wilting after 14 hours were pollinated at the base of the stigma lobes, and the lengths of pollen tubes in the grafted style were determined 4 hours later. In the controls, pollen completely compatible with both grafted and host styles was used so that there should be no inhibition, either from the tissues in which the tubes grew or from substances diffusing from the ovary. The pollen used was also com-

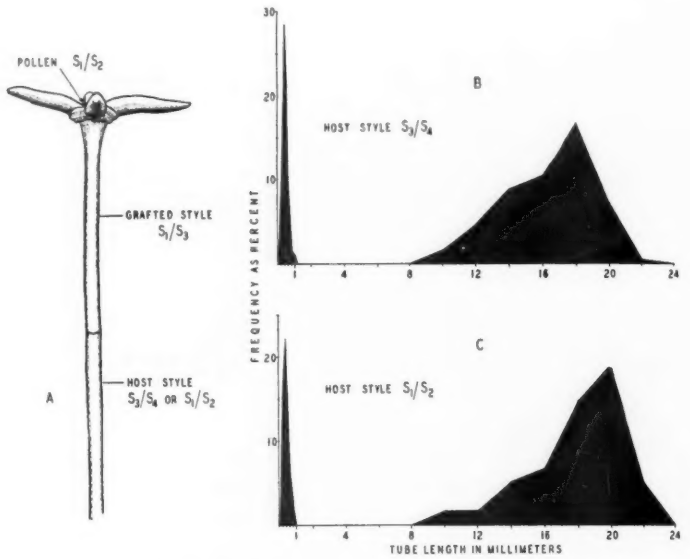



FIG. 4.—Pollen-tube growth in grafted styles. *A*, method of grafting; *B*, distribution of tubes of which half are compatible, half incompatible with grafted style in which they were growing, when host style is compatible with both types of pollen; *C*, distribution of tubes under similar conditions except that host style is incompatible with both types of pollen.

TABLE 3

GROWTH OF $S_1/S_2$ POLLEN IN $S_1/S_3$ STYLES WHICH HAD BEEN GRAFTED ON TO STYLES OF OTHER CONSTITUTIONS 14 HOURS EARLIER

| HOST STYLE | POLLEN TUBES (1 MM. OR LESS) | POLLEN TUBES (10 MM. OR MORE) |
|---|---|---|
| $S_3/S_4$ | 28 | 25 |
| | 30 | 24 |
| | 25 | 47 |
| $S_1/S_2$ | 33 | 31 |
| | 33 | 31 |
| | 31 | 34 |

pletely compatible with the grafted style, but only half the pollen (carrying $S_8$) was compatible with the host style, the other half ($S_5$) incompatible, so that none of the tubes should be inhibited by tissues in which they were growing, but half of them should be inhibited if controlling substances diffused from the ovary. The data presented in table 2 and figure 3*B*, *C* show that there is no greater tendency toward a bimodal distribution of tubes (compare with distributions illustrated in figure 4*B*, *C*) in the tests than in the controls, indicating that there is no inhibiting effect from substances diffusing from below the graft.

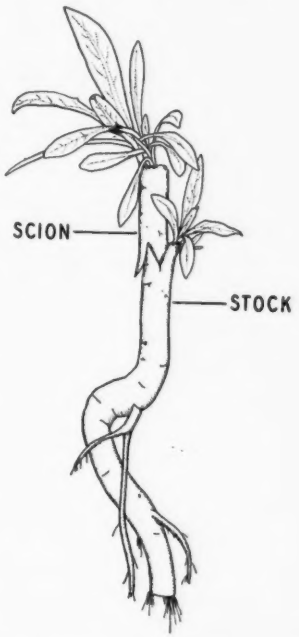



FIG. 5.—Root graft with adventitious shoots on both stock and scion.

In another experiment the growth of $S_1 / S_2$ pollen tubes was followed in $S_1 / S_3$ styles which had been grafted on to other styles (fig. 4). With no effect from grafting, $S_1$ pollen should be incompatible and $S_2$ pollen compatible with the tissues in which they were growing. In grafts on to $S_3 / S_4$ the compatible tubes should be unaffected, but the incompatible tubes, $S_1$, might be stimulated in growth by substances diffusing from compatible tissues below the graft. In grafts on to $S_1 / S_2$ the incompatible tubes should be unaffected, whereas the compatible tubes, $S_2$, might be inhibited by substances diffusing from the incompatible tissues below the graft. The data presented in table 3 and figure 4*B*, *C* again indicate that there is no observable influence from substances diffusing from below the graft.

To test for substances which might diffuse throughout the plant at very slow rates, root grafts similar to that illustrated in figure 5 were made. On two such grafts adventitious buds developed on both

stock and scion, and flowers were obtained on both simultaneously. Every test indicated that pollen-tube response was determined by the tissues in which the tubes were growing, with no effect from the other part of the graft. For example, a flower on the scion $S_2/S_4$ was pollinated with pollen from the stock $S_3/S_4$: half the pollen tubes ($S_4$) grew to less than 1 mm. in $4\frac{1}{2}$ hours and half ($S_3$) grew to 30 mm. (maximum), showing no inhibition from the stock. Self-pollinations showed that both stock (tube growth to 2 mm.) and scion (tube growth to 1.5 mm.) remained completely self-sterile. In these instances there was good union between stock and scion, and if inhibitory or stimulating substances diffused readily throughout the plant they should have been detected.

### II. Effect of light on pollen-tube growth

When plants were grown in the greenhouse in the winter and flowered by artificial lighting, there was occasionally a less sharp distinction between the growth of compatible and incompatible pollen tubes within 4 hours after pollination than is generally the case with field-grown plants. This type of response was most noticeable after a period of cloudy weather, and it was thought that the decreased light intensity might be the cause. A test of the effect of light on pollen-tube growth was made with flowers from field-grown plants in the summer of 1939.

For the principal experiment a clone of three plants of constitution $S_{12}/S_{13}$ was used. These plants were chosen because they normally flowered abundantly over a long season and because the response of incompatible pollen tubes following self-pollination was extremely regular—in nearly all flowers tested the incompatible tubes had a maximum length of 3 mm. or less.

Each of the three plants was covered in turn by a dark-house made of ply-wood, measuring 3 feet by 3 feet by 5 feet, painted black on the inside and white on the outside and ventilated top and bottom. A small amount of light diffused through the ventilators and enough light to see by was admitted when the flowers were being emasculated. Plants could be kept in the dark for 7–8 days, after which all remaining buds fell off and no further flowers would be produced for 3 weeks or longer after returning the plant to the light.

Control data were collected from each plant before it was placed in the dark and from other plants of the clone during the time one plant was in the dark. Since the controls from the three plants were uniform throughout, they are not presented separately in table 4. The tests were run so as to distinguish between possible effects of continued darkness on the pollen and stigmas separately. That is, some stigmas grown in the dark were pollinated with pollen also from the plant in the dark and others with pollen from a member of the clone kept in the light; similarly, some stigmas from a plant in the light were pollinated with pollen from the same plant and some with pollen from the plant kept in the dark. No measurable change was observed in the pollen of plants kept in the dark, even after 7 days, at which time it gave the characteristic short-tube growth in incompatible styles kept in the light, while in compatible pollinations it produced tubes of the same length as compatible pollen from plants kept in the light. Since the source of pollen made no difference in the growth response, the different types of pollinations will not be presented separately.

The styles of flowers from plants kept in the dark showed no change as measured by the response of compatible pollen tubes. Following pollination by $S_{16}/S_{17}$, the growth of pollen tubes was at the same rate in styles from plants kept in the dark as from plants kept in the light. On the other hand, styles from plants kept in the dark did show a marked change as measured by the response of incompatible pollen tubes (table 4).

No noticeable change occurs in the first 48 hours that the plant is kept in the dark. After 72 hours, however, there is a gradual increase in the length of incompatible tubes, which reaches an apparent maximum at about 144 hours. The first signs of etiolation of the young shoots generally appear after 96 hours. After 144 hours most flowers are abnormal in appearance, since the different floral parts have not grown at the usual comparative rates. All parts of the flower are considerably shortened, but especially the hypanthium, so that the style and stigma often project far above the sepal tips. In the more abnormal flowers abscission layers seem to have been formed at the top of the ovary, causing the hypanthia and styles

## TABLE 4

FREQUENCIES OF STYLES WITH POLLEN TUBES OF DIFFERENT MAXIMUM LENGTHS (INCOMPATIBLE POLLINATIONS ON $S_{12}/S_{13}$)

| LENGTH (MM.) | HOURS IN DARK | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | 120 | 144–192 |
| 0.5 | 61 | 14 | 8 | 3 | ..... | ..... | 2 |
| 1.0 | 82 | 10 | 7 | 6 | 3 | ..... | 1 |
| 1.5 | 55 | 9 | 6 | 20 | 6 | 1 | ..... |
| 2.0 | 31 | 3 | 5 | 20 | 12 | ..... | ..... |
| 3.0 | 12 | ..... | 3 | 6 | 13 | 2 | 1 |
| 4.0 | 5 | ..... | ..... | 5 | 7 | 5 | ..... |
| 5.0 | 3 | ..... | ..... | 1 | 5 | 8 | ..... |
| 6.0 | 6 | ..... | ..... | ..... | 3 | 6 | ..... |
| 7.0 | 3 | ..... | ..... | ..... | 2 | 4 | ..... |
| 8.0 | ..... | ..... | ..... | ..... | ..... | 1 | 2 |
| 9.0 | ..... | ..... | ..... | ..... | 1 | 1 | 2 |
| 10.0 | ..... | ..... | ..... | ..... | ..... | 9 | ..... |
| 11.0 | ..... | ..... | ..... | 1 | ..... | 3 | 3 |
| 12.0 | 2 | ..... | ..... | 2 | ..... | 8 | 3 |
| 13.0 | ..... | ..... | ..... | ..... | ..... | 4 | 2 |
| 14.0 | ..... | ..... | ..... | 1 | ..... | 1 | 4 |
| 15.0 | ..... | ..... | ..... | ..... | ..... | 1 | 3 |
| 16.0 | ..... | ..... | ..... | ..... | ..... | ..... | 3 |
| 17.0 | ..... | ..... | ..... | ..... | ..... | ..... | 2 |
| 18.0 | ..... | ..... | ..... | ..... | ..... | ..... | 3 |
| 19.0 | ..... | ..... | ..... | ..... | ..... | 1 | 1 |
| 20.0 | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| 21.0 | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| 22.0 | ..... | ..... | ..... | ..... | ..... | 1 | ..... |
| 23.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 24.0 | ..... | ..... | ..... | 1 | ..... | ..... | ..... |
| 25.0 | ..... | ..... | ..... | ..... | ..... | 1 | 1 |
| 26.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 27.0 | ..... | ..... | ..... | ..... | ..... | 1 | ..... |
| 28.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 29.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 30.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 31.0 | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| 32.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 33.0 | ..... | ..... | ..... | ..... | 1 | ..... | ..... |
| Mean length | 1.6 | 1.0 | 1.3 | 2.9 | 3.8 | 9.3 | 13.5 |

to break off at this level. Many of the styles with very short tubes after 144 hours or longer in the dark were from abnormal flowers.

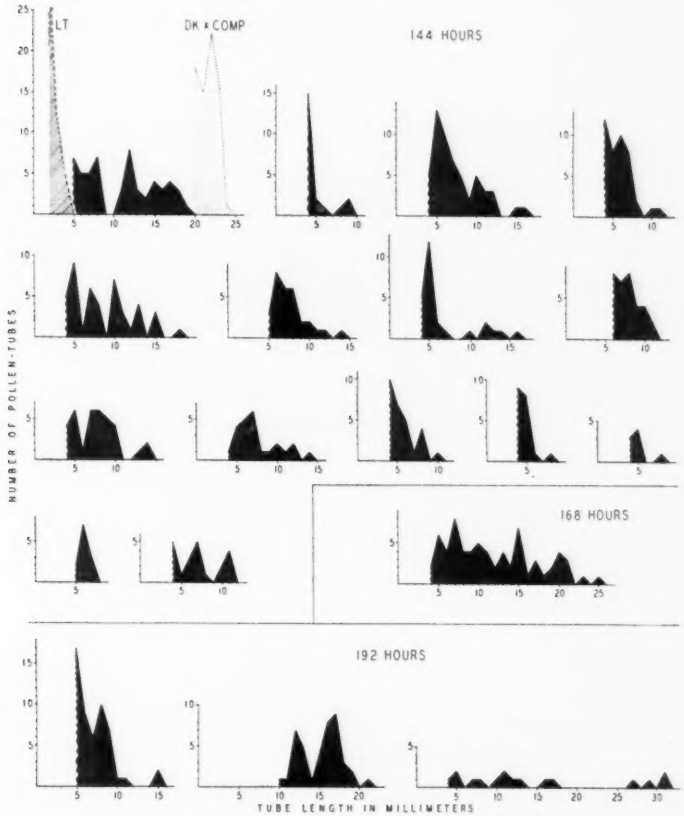



FIG. 6.—Distributions (in absolute numbers) of pollen tubes of different length, within styles of plant of $S_{12}/S_{13}$ clone. Solid black curves show distributions of incompatible pollen tubes in styles of different flowers from plant kept in dark for times indicated. Lightly shaded curve (*LT* in upper left figure) shows greater-than-average growth of incompatible pollen tubes in style from plant kept in light (also $S_{12}/S_{13}$); stippled curve (*DK*×*COMP* in same figure) shows distribution of compatible ($S_{16}/S_{17}$) pollen tubes in style ($S_{12}/S_{13}$) from plant kept in dark.

The data presented in table 4 show the measurements of only the longest pollen tube in each style. From 72 hours on, these may

be as long in some styles as compatible tubes grown for the same length of time ($4\frac{1}{2}$ hours—compatible pollen tubes generally grow 25–30 mm. in that time). But in compatible pollinations most of the pollen tubes are nearly as long as the longest, whereas comparatively few incompatible pollen tubes approach the longest in these instances. The distribution curves in figure 6 illustrate this point. These curves are based on measurements of tubes 4 mm. or longer, but since care was taken to pollinate all stigmas equally (about 300 pollen grains per stigma), the data from the different flowers should be roughly comparable.

Other plants of different constitutions also showed the same response to long periods in the dark. These plants, however, showed much greater variability between individual flowers when kept in the light, and the results are not so striking. One such example is summarized in table 5.

### Tests for seed set

The greater growth of incompatible pollen tubes in styles from plants kept in the dark is apparently due to a weakening of the incompatibility response and not to its complete disappearance. Incompatible pollinations were made as follows: six after 96 hours in the dark, four after 120 hours, nine after 144 hours, and ten after 192 hours, some being pollinated as open flowers, some as buds. Not one seed set following these twenty-nine pollinations. Control pollinations with compatible pollen were not made, so the possibility remains that failure of seed set could be due to the general weakening of the plant. In one experiment pollen tubes were allowed to grow for 8 hours instead of the usual $4\frac{1}{2}$ hours before examination, and while the experiment was not adequately controlled, the results indicated that there was little more growth in 8 hours than in $4\frac{1}{2}$, leading to the inference that the pollen tubes never reached the ovaries.

### Diffusible substances

The distal three-quarters of about ten branches of one plant of the $S_{12}/S_{13}$ clone were inclosed in a dark chamber, the remainder of the plant being exposed to light. Under these conditions the

TABLE 5

FREQUENCIES OF STYLES WITH POLLEN TUBES OF DIFFERENT MAXIMUM LENGTHS (INCOMPATIBLE POLLINATIONS ON $S_2/S_3$)

| LENGTH (MM.) | HOURS IN DARK | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 | 216 |
| 0.5 | 3 | 8 | 3 | 9 | ..... | 4 | 3 | 1 | ..... | ..... |
| 1.0 | 6 | 3 | 2 | 8 | 1 | 1 | ..... | 3 | ..... | ..... |
| 1.5 | 5 | 3 | 3 | 5 | 6 | 4 | 1 | ..... | ..... | ..... |
| 2.0 | 6 | ..... | 2 | 8 | 2 | 1 | 3 | ..... | ..... | 1 |
| 3.0 | 6 | ..... | 1 | 2 | ..... | 1 | ..... | ..... | ..... | ..... |
| 4.0 | 7 | ..... | 2 | 1 | 2 | ..... | 2 | ..... | ..... | ..... |
| 5.0 | 5 | ..... | 1 | ..... | ..... | 1 | ..... | 1 | ..... | ..... |
| 6.0 | 5 | ..... | 1 | ..... | ..... | ..... | 4 | 6 | ..... | 1 |
| 7.0 | 5 | ..... | ..... | ..... | 2 | 1 | 2 | 1 | ..... | ..... |
| 8.0 | 1 | ..... | ..... | 1 | ..... | 2 | ..... | 1 | ..... | ..... |
| 9.0 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | 2 | ..... | ..... |
| 10.0 | 2 | ..... | ..... | ..... | ..... | ..... | 1 | 2 | ..... | ..... |
| 11.0 | ..... | ..... | ..... | ..... | ..... | 1 | 1 | 1* | ..... | 1 |
| 12.0 | ..... | ..... | ..... | ..... | 1 | ..... | 2 | ..... | ..... | 2 |
| 13.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 2 | 1 | 1 |
| 14.0 | 1 | ..... | ..... | ..... | ..... | ..... | 2 | 1 | ..... | 1 |
| 15.0 | ..... | ..... | 1 | ..... | ..... | ..... | 1 | ..... | 1 | 1 |
| 16.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 17.0 | 1+1* | 1 | ..... | ..... | ..... | ..... | ..... | 1* | ..... | ..... |
| 18.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 19.0 | 1 | ..... | ..... | ..... | ..... | ..... | 1 | ..... | ..... | 1 |
| 20.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1 | 1 |
| 21.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 22.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| 23.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 24.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1 | ..... | ..... |
| 25.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1* | 1* | 1 |
| 26.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1* | ..... | ..... |
| 32.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| 45.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1* |
| Mean length | 4.9 | 1.7 | 3.1 | 1.6 | 3.0 | 2.8 | 7.0 | 9.6 | 18.0 | 17.7 |

* Abundant pollen-tube growth resembling compatible pollinations.

branches kept in the dark flowered almost indefinitely, but without giving any increased growth of incompatible pollen tubes (table 6).

Another plant (constitution $S_5 / S_7$) was kept in the dark for 120 hours, after which most of the plant was exposed to the light, with a few branches continued in the dark. The dark effect was still pronounced after the plant had been returned to the light for 24 hours, but after 48 hours the incompatibility response was back to normal. Unfortunately there were almost no flowers produced on

TABLE 6

FREQUENCIES OF STYLES WITH POLLEN TUBES OF DIFFERENT MAXIMUM LENGTHS (INCOMPATIBLE POLLINATIONS ON $S_{12}/S_{13}$)

| LENGTH (MM.) | BRANCHES IN LIGHT | BRANCHES IN DARK (TIME IN HOURS) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24 | 72 | 120 | 144 | 168 | 192 | 216 | 288 |
| 0.5 | 19 | 3 | 3 | ..... | ..... | 1 | 1 | 3 | ..... |
| 1.0 | 9 | ..... | ..... | 1 | ..... | ..... | ..... | ..... | 4 |
| 1.5 | 3 | 1 | 1 | ..... | ..... | ..... | 1 | ..... | 1 |
| 2.0 | 1 | ..... | ..... | 3 | ..... | ..... | ..... | ..... | ..... |
| 3.0 | 2 | ..... | ..... | ..... | 1 | ..... | ..... | ..... | ..... |
| 4.0 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 5.0 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 6.0 | 1 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 1 |
| Mean length | 1.2 | | | 1.4 | | | | | |

the branch continued in the dark after the first day, but recovery was apparently as rapid as on the branches in the light (table 7).

It has been shown in part I of this paper that the specific inhibitory factors responsible for the incompatibility reaction do not diffuse from tissues of one constitution to those of another. The two experiments just described indicate that substances diffusing from branches kept in the light prevent branches kept in the dark from showing the typical dark reaction. It is probable that these substances are not the specific incompatibility factors, since these are known not to diffuse readily. It is possible that some general precursor of all specific factors is made only in sunlight, but it is equally

TABLE 7

FREQUENCIES OF STYLES WITH POLLEN TUBES OF DIFFERENT MAXIMUM LENGTHS (INCOMPATIBLE POLLINATIONS ON $S_5/S_7$)

| Length (mm.) | Entire plant in dark (time in hours) | | | | | After 120 hours in dark | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Branches returned to light (time in hours) | | | Branches retained in dark (time in hours) | | |
| | 24 | 48 | 72 | 96 | 120 | 24 | 48 | 72 | 24 | 48 | 72 |
| 0.5 | 2 | | | | | | 2 | 2 | | 1 | |
| 1.0 | 7 | 2 | 1 | | | | 6 | 4 | | | |
| 1.5 | 5 | 3 | | | 2 | | 5 | 5 | | | |
| 2.0 | 4 | 4 | 2 | 1 | | | 3 | 4 | | | |
| 3.0 | 2 | 2 | 5 | 1 | | 2 | 1 | 4 | | | 1 |
| 4.0 | | 1 | 1 | | | 3 | | 1 | | | |
| 5.0 | | 1 | | 2 | 1 | 2 | | | | | |
| 6.0 | | | | 1 | 2 | 3 | | | 2 | | |
| 7.0 | | 1 | 2 | 3 | | 1 | | | 1 | | |
| 8.0 | | | | | | 1 | | | | | |
| 9.0 | | | 1 | 3 | 1 | 3 | | | 1 | | |
| 10.0 | | | | 1 | 1 | | | | | | |
| 11.0 | | | 2 | | 3 | | | | | | |
| 12.0 | | | | 1 | 1 | 2 | | | | | |
| 13.0 | | | 2 | | | | | | | | |
| 14.0 | | | | 2 | | | | | 1 | | |
| 15.0 | | | | | 1 | | | | | | |
| 16.0 | | 1 | | 1 | | | | | | | |
| 17.0 | | | | 1 | | | | | | | |
| 18.0 | | | | | | | | | | | |
| 19.0 | | | | 1 | | | | | | | |
| 20.0 | | | | 2 | | | | | | | |
| 21.0 | | | | 2 | | | | | | | |
| 25.0 | | | 1 | | | | | | | | |
| Mean length | 1.5 | 3.5 | 7.0 | 11.5 | 8.3 | 6.6 | 1.4 | 1.8 | 8.5 | 1.8 | |

possible that the dark reaction is due to a general physiological weakening of the plant, which is overcome by exposing part of the plant to light. Unfortunately the root grafts described earlier had been discarded, so that it has not yet been possible to test directly for the specificity of the dark reaction.

Buds removed individually from the plant in the dark and kept for 24 hours in a dark moist chamber, either in water or in a 2 per cent sugar solution, continued to show the typical dark reaction. If as little as 1 per cent (by dry weight) of ground-up leaves was added either to the water or sugar solution, the buds wilted and failed to open. When stigmas of these wilted flowers were pollinated, the pollen-tube growth was atypical, the tubes having nearly twice the diameter of tubes in fresh styles. Flowers on cut branches reacted in the same way to the addition of leaf extract.

## Summary

1. The characteristic features of self-sterility in *Oenothera organensis* are discussed. The genetic basis of self-sterility is a series of some forty-five allelomorphs of one gene. The reaction of the pollen is a gametophytic character, governed solely by the genetic constitution of the pollen itself, with no influence from the second allelomorph carried by the sporophyte plant on which it is borne. Incompatible pollen tubes differ from compatible in extent of growth, and in no other discernible morphological character. Self-sterility is apparently completely effective in preventing seed formation in this species: there is no bud fertility or end-season fertility and all usual methods of obtaining self-pollinated seeds have failed. The incompatibility response is due to an inhibition of pollen-tube growth, which is not governed by diffusible substances but seems to be a reaction between the pollen tube and the tissue in which it is growing.

2. The extent of growth of incompatible pollen tubes varies with the particular sterility allelomorph present in the pollen, with other undetermined genetic factors, and with certain environmental conditions. The most pronounced effect of changes in environment is that resulting from prolonged absence of light. When plants have

been kept in the dark for 3 days or longer, their styles permit much longer incompatible tube growth than occurs in flowers from plants kept in the light. The maximum effect is noticed after 5 days, after which the plant develops signs of general weakness and usually stops flowering after the 7th or 8th day. When parts of the plants are covered and the remainder left exposed to light, flowers from branches kept in the dark do not permit increased growth of incompatible pollen tubes. It has not been determined that the diffusion from branches in the light to those in the dark involves substances specifically connected with the incompatibility reaction. It is equally likely that the greater growth of incompatible pollen tubes in flowers from the dark is due to a general physiological debility of the plant, which can be overcome by leaving part of the plant exposed to light.

California Institute of Technology
Pasadena, California

## LITERATURE CITED

1. East, E. M., Genetical aspects of self- and cross-sterility. Amer. Jour. Bot. **10**:468–473. 1923.
2. ———, Self-sterility. Bibliogr. Genet. **5**:331–370. 1929.
3. ———, Norms of pollen-tube growth in incompatible matings of self-sterile plants. Proc. Nat. Acad. Sci. **20**:225–230. 1934.
4. East, E. M., and Mangelsdorf, A. J., A new interpretation of the hereditary behavior of self-sterility in plants. Proc. Nat. Acad. Sci. **11**:166–171. 1925.
5. Emerson, S., The genetics of self-incompatibility in *Oenothera organensis*. Genetics **23**:190–202. 1938.
6. ———, A preliminary analysis of the *Oenothera organensis* population. Genetics **24**:524–537. 1939.
7. Sears, E. R., Cytological phenomena connected with self-sterility in the flowering plants. Genetics **22**:130–181. 1937.
8. Yasuda, S., An experiment to graft the style upon the ovary in *Petunia violacea*. Proc. Imp. Acad. Tokyo **7**:72–75. 1931.
9. ———, Studies pertaining to self-incompatibility in some plants of Solanaceae. III. Physiological aspects of self-incompatibility in *Petunia violacea*. Ann. Rept., Saito ho-on Kai (Saito Graduate Foundation). No. 9. 1933.
10. ———, Physiological research on self-incompatibility in *Petunia violacea*. Bull. Imp. Coll. Agr. Forestry, Morioka, Japan. No. 20. 1934.

# EFFECT OF POTATO EXTRACTS ON GROWTH OF PHYCOMYCES

WILLIAM J. ROBBINS AND KARL C. HAMNER[1]

(WITH THREE FIGURES)

In previous papers ROBBINS (**7, 8**) concluded that one or more growth substances in addition to thiamin are concerned in the development of *Phycomyces blakesleeanus*. This substance, referred to as factor Z, is produced by *Phycomyces* (**9**) but not in amounts adequate for its maximum development, especially at or near 25° C.

Factor Z is present in various natural products, including the tubers of the white potato, from which it can be extracted with water or dilute alcohol. In response to its presence, the spore germination, early mycelial growth, and gametic reproduction of *Phycomyces* are increased in a medium of mineral salts, sugar, asparagine, and thiamin. From various observations it seemed probable that factor Z is multiple and does not consist of a single substance. In the present paper evidence on the multiple nature of factor Z is reported. There appear to be at least two substances composing it. If an aqueous solution containing factor Z is shaken with charcoal, one fraction is adsorbed by the charcoal and another is not; it remains in the aqueous filtrate following filtration of the mixture.

## Material and methods

Two potato extracts were prepared.[2] Extract $P_2$ was obtained as follows. Six kg. fresh potatoes were ground in a food chopper, after removing the peel. The pulp was covered with 2.5 liters of 60 per cent methanol and allowed to stand for 18 hours at room temperature. The extract was strained through cheesecloth, centrifuged, and the supernatant liquid heated to boiling. The coagulum which formed was removed by centifuging, the liquid evaporated to 150 ml. and

[1] Assistance in this work was furnished by the personnel of Works Projects Administration Official Project 65-1-97-23 W.P. 5. The work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Additional cost of publication was borne by the authors.

[2] The assistance of Frederick Kavanagh aided in preparing these extracts.

extracted twice with n-butanol. The aqueous residue was evaporated and made up to 125 ml. It was a dark brown solution and contained 61.8 per cent solids. One ml. represented the extract of 48 gm. of potatoes.

Extract $P_{17}$ was prepared as follows. Thirty kg. of peeled and sliced potatoes were covered with 50 per cent methanol for 18 hours. The liquid was decanted, the starch allowed to settle, and removed. The alcoholic liquid was then evaporated to about 500 ml. and allowed to crystallize in the refrigerator. The crystals were removed, dissolved in water, and recrystallized. The two supernatant liquids, the one remaining after the first crystallization and the one remaining after the second, were combined and treated with an equal volume of methanol and further precipitation completed in the cold. The precipitate was removed, dissolved in water, and reprecipitated. The liquid fractions remaining after the two precipitations were made up to 70 per cent methanol. The precipitate which formed was removed, dissolved in water, and reprecipitated. The several precipitates were discarded, but all the liquid fractions were combined, evaporated nearly to dryness, and then made up to 100 ml. with water. This dark brown liquid contained 67.6 per cent solids, and 1 ml. represented the extract from 300 gm. potatoes.

In culturing the fungus, each liter of the basal solution (solution I) used in these experiments contained 50 gm. dextrose, 2 gm. asparagine, 1.5 gm. $KH_2PO_4$, 0.5 gm. $MgSO_4 \cdot 7\ H_2O$, 0.5 mg. thiamin, and the following trace elements: 0.005 ppm B, 0.02 ppm Cu, 0.1 ppm Fe, 0.01 ppm Ga, 0.01 ppm Mn, 0.01 ppm Mo, and 0.09 ppm Zn. All chemicals were of chemically pure grade; the dextrose was Corn Products Company chemically pure; the thiamin, Merck's synthetic; the asparagine was purified by precipitation from alcohol. In testing the effect of various extracts on spore germination, growth, and zygote formation, various amounts of the extracts were added to aliquots of the basal solution (solution I) and the response of the fungus to these additions of the extract was followed. All solutions were sterilized at 110° C. just prior to inoculation.

For spore germination tests, agar was added to the liquid media to make a 1 per cent mixture which solidified when poured into petri dishes. Drops of a spore suspension of *Phycomyces* were placed on

the agar and the percentage of germinated spores counted 12–15 hours after incubation at 23°–25° C.

For growth studies, 25 ml. of solution were used in 125 ml. Erlenmeyer flasks. A drop of a spore suspension was used as inoculum and the cultures were incubated approximately 72 hours at 23°–25° C. The mycelium was filtered into Gooch crucibles, washed, dried at 100° C., and weighed. The results are recorded in milligrams of dry mycelium.

For some studies of gametic reproduction the solutions were poured into petri dishes, 12.5 ml. per dish, and the liquid inoculated with a drop of a spore suspension of both the plus and minus strains. The cultures were incubated at 23°–25° C. and the production of progametes and zygotes noted. In other studies a drop of the spore suspensions of each of the plus and minus strains was placed at opposite ends of a diameter on an agar plate and the production of progametes and zygotes noted after incubation for 8 days.

## Experimentation

EFFECT OF POTATO EXTRACT.—Various quantities of an extract of potato ($P_2$) were added to aliquots of solution I containing 1 per cent Difco agar. Twenty-five ml. quantities of each resulting solution were poured into sterile petri dishes. Drops of a spore suspension of the plus strain of *Phycomyces* were placed on the medium and the plates incubated for 15 hours at 23°–25° C. The experiment was performed in duplicate and the results were as follows:

| Dry matter of potato extract $P_2$ per plate (mg.) | Percentage germination | Dry matter of potato extract $P_2$ per plate (mg.) | Percentage germination |
|---|---|---|---|
| 0 | 0.36 | 31 | 38.5 |
| 1.5 | 0.7 | 78 | 72.5 |
| 7.7 | 2.2 | 155 | 90.0 |
| 15.0 | 4.0 | | |

It is evident that this extract had a marked effect in increasing the spore germination of *Phycomyces* at 23°–25° C. in an agar medium containing mineral salts, sugar, asparagine, and thiamin. Potato extract also had a distinct effect in increasing the rate of growth of the mycelium of *Phycomyces*. To 25 ml. quantities of solution I various quantities of extract $P_2$ were added and each resulting solution inoculated with a drop of a spore suspension of the plus strain

of *Phycomyces*. After 3 days of incubation at 23°–25° C. the dry weight of the mycelium was determined with the following results (also shown in figure 1):

| $P_2$ ADDED TO EACH FLASK (ML.) | DRY MYCELIUM PRODUCED (MG.) | $P_2$ ADDED TO EACH FLASK (ML.) | DRY MYCELIUM PRODUCED (MG.) |
|---|---|---|---|
| 0.000 | 1.8 | 0.05 | 88.9 |
| 0.001 | 2.3 | 0.1 | 113.8 |
| 0.005 | 8.9 | 0.2 | 129.2 |
| 0.01 | 27.8 | 0.5 | 87.4 |
| 0.02 | 54.1 | | |

One ml. of extract $P_2$ contained 618 mg. dry matter.

In another experiment designed to test its effect on the gametic reproduction of *Phycomyces*, various quantities of $P_2$ were added

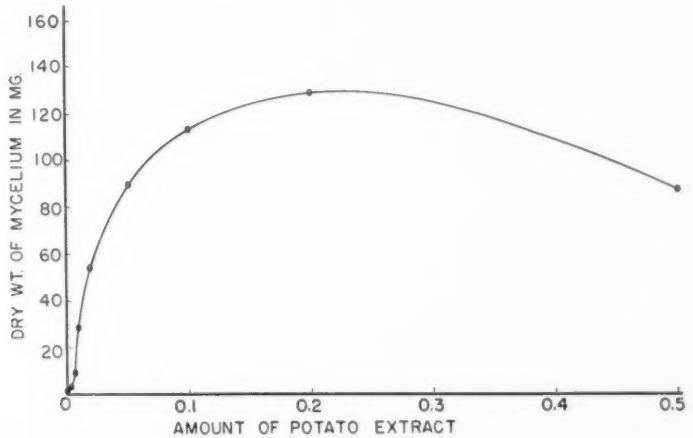



FIG. 1.—Effect of potato extract (amount used expressed in ml. of extract per plate) upon early growth of *Phycomyces* at 23°–25° C. in a solution of mineral salts, sugar, asparagine, and thiamin.

to solution I containing 1 per cent Difco agar purified by extraction with 5 per cent aqueous pyridine (7). Twenty-five ml. of these media were poured into petri dishes and inoculated at opposite ends of a diameter with plus and minus strains of *Phycomyces*. The experiment was set up in quadruplicate. The cultures were incubated at 23°–25° C. for 8 days and then observations on gametic reproduction were made. The results are shown in table 1 and figure 2.

TABLE 1

EFFECT OF POTATO EXTRACT $P_2$ ON PRODUCTION OF PROGAMETES AND ZYGOTES BY PHYCOMYCES

| EXTRACT $P_2$ ADDED PER PLATE (ML.) | DRY MATTER ADDED PER PLATE (MG.) | LENGTH OF LINE PROGAMETES IN CM. ON PLATES 9 CM. IN DIAMETER | NO. ZYGOTES PER PLATE |
|---|---|---|---|
| 0.25 | 155 | 9.0 | 143 |
| 0.20 | 124 | 9.0 | 175 |
| 0.15 | 93 | 9.0 | 258 |
| 0.10 | 62 | 9.0 | 236 |
| 0.075 | 46 | 9.0 | 87 |
| 0.050 | 31 | 9.0 | 50 |
| 0.040 | 25 | 9.0 | 38 |
| 0.030 | 18.6 | 9.0 | 32 |
| 0.020 | 12.4 | 3.2 | 1 |
| 0.015 | 9.3 | 1.7 | None |
| 0.010 | 6.2 | 0.7 | None |
| 0.005 | 3.1 | None | None |
| 0.0025 | 1.5 | None | None |
| 0.00125 | 0.7 | None | None |
| 0.0005 | 0.4 | None | None |
| None | None | None | None |

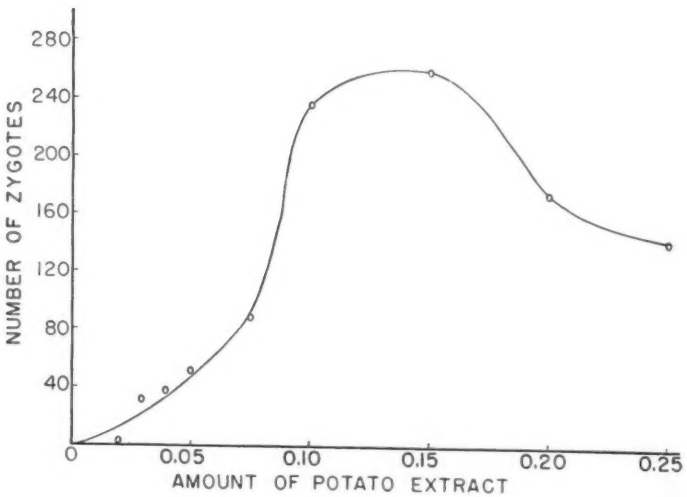



FIG. 2.—Effect of potato extract (amount used expressed in ml. of extract per plate) upon gametic reproduction of *Phycomyces* on agar medium containing mineral salts, sugar, asparagine, and thiamin.

Growth was least vigorous in the plates to which no potato extract was added. In those plates where no progametes occurred the mycelium covered the surface of the medium and abundant sporangiophores and sporangia were produced.

FRACTIONATION OF POTATO EXTRACT.—In the course of observations on the effect of various extracts from several sources, including those prepared from potato, upon the development of *Phycomyces*, there were indications that some extracts resulted in increased growth but not gametic reproduction, while others appeared to result in an increase of both growth and gametic reproduction. It seemed desirable, therefore, to fractionate the potato extract and determine if possible whether more than one factor present in potato extract was concerned in its resultant effects.

A quantity of an extract of potato ($P_{17}$) was treated with ethanol to form a 93 per cent alcoholic solution. The precipitate which formed was removed, dissolved in water and treated in slightly acid solution with norit A, and filtered. The filtrate was evaporated to dryness in partial vacuum and taken up with $H_2O$. This solution was designated $P_{17}B$. The norit, which remained on the filter, was shaken with 5 per cent aqueous pyridine and the resulting solution freed from pyridine by evaporation to dryness in partial vacuum. The residue was taken up with $H_2O$ and designated $P_{17}A$. The original filtrate from the treatment of the $P_{17}$ extract with 93 per cent alcohol was evaporated to dryness in partial vacuum, taken up with water and treated with norit in slightly acid solution. This mixture was then filtered, and the filtrate labeled $P_{17}D$. The norit, which remained on the filter, was then treated with 5 per cent aqueous pyridine, filtered, and the resulting solution freed from pyridine by evaporation and designated $P_{17}C$. These four fractions were diluted with distilled water so that 1 ml. was equivalent to the extract from 50 gm. of fresh potato. Each of the four extracts was tested for activity as it affected growth of the fungus in a manner similar to that already described for potato extract $P_2$.

For brevity, these solutions are designated respectively as A, B, C, and D. Extracts A, B, and C showed slight activity; extract D was about two-thirds as active in promoting development of the fungus as the original potato extract $P_{17}$. On the basis of a comparison of the dry matter in the original extract with the total of that in the four

fractions, it was found that some 15 per cent of the original dry matter could not be accounted for.

On the assumption that this material had been adsorbed by the norit and not freed by the pyridine treatment, the norit from which fraction $P_{17}A$ was obtained and that from which $P_{17}C$ was obtained were each treated with ammoniacal acetone (60 per cent acetone and 2.5 per cent ammonia). Each norit and acetone mixture was shaken for several hours and filtered. The norit was extracted in this manner with ammoniacal acetone three times and the acetone extracts from each individual lot of norit combined, evaporated to dryness in partial vacuum, and the residues taken up in enough water so that 1 ml. of solution was equivalent to 50 gm. of fresh potato. The two solutions thus obtained, one from the norit of fraction $P_{17}A$ and one from the norit of fraction $P_{17}C$, are designated Aa and Ca, respectively. Aa had very little effect in promoting development of *Phycomyces* and Ca was decidedly more active than C, although considerably less so than the original extract $P_{17}$ or fraction D. It appeared from these results that two active fractions had been secured from potato: one (Ca) was adsorbed on charcoal from a weakly acid solution and freed from the charcoal by ammoniacal acetone; the other (fraction D) was in the charcoal filtrate. Fraction D was again treated with norit until all color was removed and the colorless filtrate designated fraction Dr. Both fractions, Ca and Dr, were adjusted with distilled water so that 1 ml. was equivalent to the extract from 50 gm. potato. Fraction Ca contained 0.51 per cent solids and Dr 5.71 per cent.

EFFECT OF FRACTIONS CA AND DR ON GROWTH AND GAMETIC REPRODUCTION.—Preliminary experiments indicated that Ca affected mycelial growth but had little effect upon the formation of zygotes, while Dr had a greater effect on growth and also increased gametic reproduction.

The effect of the extracts on growth and gametic reproduction was determined by the addition of various quantities of Ca and Dr, both separately and in combination, to solution I. For determining the effect on gametic reproduction, two methods were employed. In one, duplicate petri dishes each containing 12.5 ml. of solution I and inoculated with a drop of the plus and the minus strains were used. In the other duplicate petri dishes each containing 25 ml. of solution I to which had been added 1 per cent agar which had been purified

by extraction with pyridine were employed. The agar plates were inoculated with the plus and minus strains at opposite ends of a diameter. In studying the effects of the extracts on growth, the dry weight of the mycelium was determined after 3 days' incubation by the method described in the use of the extract $P_2$. All cultures were incubated at 23°–25° C.

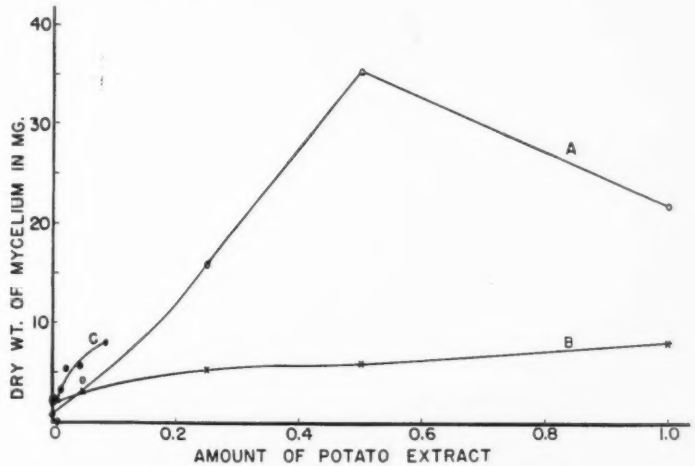



FIG. 3.—Effect of fractions of potato extract on early growth of *Phycomyces: A*, charcoal filtrate; *B*, charcoal adsorbate; *C*, effectiveness of charcoal adsorbate expressed in terms of dry matter added, the units on the abscissa conforming with equivalent amounts of dry matter of charcoal filtrate as represented in *A*.

Fractions Ca and Dr both brought about an increase in the rate of growth of *Phycomyces* when either was added to solution I (fig. 3). The Dr fraction produced more effect per ml. of extract used, but since this fraction contained more dry matter per ml. than Ca, both fractions were about equally effective on the basis of dry matter added. An effect was noted with the addition of as little as 25 gamma of dry matter of fraction Ca.

From a comparison of the effects of the two fractions, when used alone, with that of their effects when used in combination (table 2), it would appear that each contained some promotive factor or factors which was more effective in the presence of the other than when used

alone. For example, the presence of 1 ml. of Dr resulted in 22.1 mg. of mycelium and the presence of 0.005 ml. of Ca in 2.1 mg. of growth as compared with 1.4 mg. produced when neither was present. The additions of both these (that is, 1 ml. of Dr and 0.005 ml. of Ca) to the same flask resulted in 81.1 mg. of growth. Numerous other such examples may be found in table 2. Thus, addition of small amounts of both fractions to the same culture resulted in far greater mycelial growth than might be expected on the basis of a consideration of the individual effects produced by either fraction alone.

TABLE 2

COMBINED AND INDIVIDUAL EFFECTS OF FRACTIONS CA AND DR OF POTATO EXTRACT ON GROWTH OF PHYCOMYCES IN BASAL SOLUTION CONTAINING THIAMIN. MG. DRY WEIGHT OF MYCELIUM

| Extract Ca per 25 ml. medium (ml.) | Ml. of fraction Dr per 25 ml. medium | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | 0.5 | 0.25 | 0.05 | 0.005 | 0 |
| 1.0 | 35.7 | 47.1 | 30.8 | 10.6 | 7.9 | 8.2 |
| 0.5 | 68.1 | 48.3 | 33.7 | 11.8 | 5.5 | 5.9 |
| 0.25 | 59.5 | 84.3 | 42.5 | 10.9 | 4.3 | 5.2 |
| 0.05 | 65.2 | 81.5 | 24.2 | 9.5 | 3.2 | 2.9 |
| 0.005 | 81.1 | 77.9 | 22.7 | 11.8 | 1.8 | 2.1 |
| 0.000 | 22.1 | 35.8 | 16.0 | 3.9 | 0.1 | 1.4 |

The effect of the combined extracts on gametic reproduction also was greater than that of either alone. Few progametes developed in the plates containing the liquid medium to which the Ca fraction was added and none on the check plates (table 3). In those to which 1 ml. of the Dr fraction was added, the surface of the mycelium was nearly covered with progametes. The quantity of progametes, however, was increased by the addition of both fractions. In those containing 0.5 ml. of the Dr fraction scattered groups of progametes appeared, and the further addition of 0.25 ml. and 0.5 ml. of fraction Ca resulted in marked increase in their production. The character of the mycelial growth in the plates with fraction Ca differed from that in those containing the Dr fraction. In the latter more colonies developed with a dry surface; in the former the colonies were fewer and larger and remained submerged for a longer period.

On the agar plates also the production of progametes was greater

where the two fractions were combined than where they were used singly. However, more progametes were formed on the agar with the larger quantities of fraction Ca than in the liquid plates, suggesting that the agar may have contained some of the Dr factor, not removed in the process of purification by pyridine.

TABLE 3

EFFECT OF TWO FRACTIONS OF POTATO EXTRACT ON FORMATION OF PROGAMETES IN LIQUID MEDIA. RELATIVE PRODUCTION OF PROGAMETES PER PLATE

| ML. OF FRACTION CA PER 25 ML. MEDIUM | ML. OF FRACTION DR PER 25 ML. MEDIUM | | | | | |
|---|---|---|---|---|---|---|
| | 1.0 | 0.5 | 0.25 | 0.05 | 0.005 | 0 |
| 1.0 | Heavy | Heavy | Few | Few | Very few | Very few |
| 0.5 | Heavy | Heavy | Few | Few | Few | Few |
| 0.25 | Heavy | Heavy | Few | Few | Few | Very few |
| 0.05 | Heavy | Scattered groups | Few | Few | Few | Few |
| 0.005 | Heavy | Scattered groups | Few | ..... | ..... | None |
| 0 | Heavy | Scattered groups | Few | Few | Very few | None |

TABLE 4

EFFECT OF TWO FRACTIONS OF POTATO EXTRACT ON PERCENTAGE OF SPORE GERMINATION BY PHYCOMYCES

| ML. OF FRACTION DR PER 25 ML. OF MEDIUM | ML. OF FRACTION CA PER 25 ML. OF MEDIUM | | | |
|---|---|---|---|---|
| | 1.0 | 0.5 | 0.25 | 0 |
| 1.0 | 62.4 | 71.0 | 64.1 | 31.8 |
| 0.5 | 50.6 | 47.9 | 42.2 | 15.2 |
| 0.25 | ..... | ..... | ..... | 26.0 |
| 0 | 10.9 | 6.0 | 0.5 | 1.2 |

The two fractions when used together evidenced more than an additive effect upon germination of the spores of *Phycomyces*. In this instance Ca and Dr extracts were freshly prepared. Drops of a spore suspension were placed on solution I containing 1 per cent Difco agar to which various quantities of the two extracts were added (table 4). The plates were incubated at 23°–25° C. and the percent-

age of germination determined after 12 hours of incubation. The plates containing the fraction Dr exhibited a higher percentage of germination than those containing equivalent volumes of Ca. When the two fractions were combined the effect was considerably greater than would be expected on an additive basis.[3]

INFLUENCE OF ANTI GRAY-HAIR FACTOR.—Through the courtesy of Dr. AGNES MORGAN, of the University of California, a concentrate of the anti gray-hair factor prepared from yeast was received. Added to solution I this concentrate had a marked effect in stimulating the early growth of *Phycomyces*. One mg. of the concentrate in 25 ml. of

TABLE 5

EFFECT OF ANTI GRAY-HAIR FACTOR CONCENTRATE AND FRACTION DR OF POTATO EXTRACT ON GROWTH OF PHYCOMYCES. MG. DRY WEIGHT OF MYCELIUM

| AMOUNT OF ANTI GRAY-HAIR FACTOR IN GM. SOLIDS ADDED TO 25 ML. OF MEDIUM | ML. OF FRACTION DR PER 25 ML. MEDIUM | | |
|---|---|---|---|
| | 1.0 | 0.25 | 0 |
| 0.04 | 43.3 | 29.1 | 21.7 |
| 0.02 | 140.9 | 111.8 | 32.2 |
| 0.004 | 140.3 | 113.3 | 42.9 |
| 0.001 | 129.7 | 58.4 | 3.9 |
| 0 | 22.1 | 16.0 | 2.3 |

solution I was sufficient to produce a definite increase in growth; larger quantities produced greater increases (table 5), the increases becoming relatively smaller with the largest amounts used. Although the effect of the anti gray-hair factor concentrate increased the growth of *Phycomyces* in the presence of thiamin, it had relatively little effect upon the gametic reproduction in liquid plates. In fact the general effect of the concentrate upon development of *Phycomyces* resembled that of the charcoal adsorbate prepared from potato tubers. The similarity between the effects of the anti gray-hair factor concentrate and the Ca fraction was confirmed by studying the effect of combinations of the concentrate with each of the two fractions of the potato extract. When various amounts of frac-

[3] The assistance of Leon Grodsinsky in performing this experiment is gratefully acknowledged.

tion Ca—ranging from 0.005 to 1 ml. per flask—were added to solution I containing the anti gray factor, no marked increase in growth of *Phycomyces* occurred. On the other hand, combinations of the concentrate with the fraction Dr gave high yields, considerably larger than could be accounted for on an additive basis (table 5). Furthermore, the anti gray-hair factor concentrate with the fraction Dr markedly favored gametic reproduction, some combinations resulting in thousands of zygotes with black appendages. The production of progametes with solutions supplemented with the anti gray concentrate alone was relatively scant. Although additions of sufficient extract Dr alone caused profuse production of progametes, no zygotes with appendages were formed.

ATTEMPTS AT IDENTIFICATION OF EFFECTIVE FRACTIONS OF EXTRACTS.—It was thought that the factor Dr might be m-inositol, which has been demonstrated by EASTCOTT (**2**) to be a part of the bios complex and found by BUSTON and PRAMANIK (**1**) to be one of the two growth substances required by *Ashbya gossypii;* however, the addition of 20 mg. per liter of inositol to solution I containing 0.005–0.25 ml. of extract Ca per culture had no effect upon the development of *Phycomyces*. Inositol alone added to solution I was ineffective.

It was reported previously (**8**) that vitamin $B_2$, vitamin $B_6$, and nicotinic acid did not increase growth of *Phycomyces* in the presence of thiamin. A combination of these three growth substances together with adenine sulphate and $\beta$ alanine used in the presence of thiamin and extract Ca was ineffective. The following mixture was added to each flask containing 25 ml. of solution I:

| | Mg. |
|---|---|
| Vitamin $B_2$ (lactoflavin) | 0.0038 |
| Vitamin $B_6$ | 0.005 |
| Nicotinic acid | 0.000005 |
| $\beta$ alanine | 0.005 |
| Adenine sulphate | 0.005 |

The amounts of m-inositol and other growth substances listed were arbitrarily selected. Other quantities might act differently, although we have no reason at present to anticipate such an eventuality.

## Discussion

Interpretation of the effects of extracts of natural products upon development of an organism depends in part upon the conditions under which the extracts are used. If the basic medium already contains luxus amounts of the essential mineral salts, as well as available carbon and nitrogen compounds, any benefit from the extracts cannot be ascribed to an addition to the supply of these classes of materials. Of course, not all mineral salts and not all compounds of carbon and nitrogen are equally available. Iron, for example, may be present in large amounts and the organism still suffer from a deficiency because the form of the iron is relatively unavailable. The quality of nutrients and major foods as well as their quantity must therefore be borne in mind. Furthermore, the total amount of growth must be considered. None of the nutrients or foods may limit growth in a given medium if the period of growth is short and the total tissue which develops is small, yet with longer time and greater growth one or more of these materials may become limiting factors.

We believe that the medium used in our experiments contains mineral nutrients, foods, and thiamin in amounts exceeding the needs of the organism for the period of the experiments. This is obviously true for the experiments on spore germination where the demand for nutrients and foods is distinctly limited. A similar condition exists for a 3-day growth period during which but a few milligrams of mycelium are produced in the basic solution. Furthermore, extremely small amounts of the extract (25 gamma of Ca) were found to be effective, which also would suggest the action of a growth substance. Considerations of this character, as well as others previously discussed (**7, 8, 9**), have led to the assumption that an unidentified growth substance or substances, factor Z, is present in potato extracts and is responsible for their beneficial action.

If this assumption is correct, then it would seem to follow that factor Z consists of at least two growth substances, one which can be adsorbed on charcoal and one which after filtration would be present in the filtrate. It is proposed to refer to the former as factor $Z_1$ and to the latter as factor $Z_2$. Unless the existence of two factors is assumed, it is difficult to explain why the charcoal adsorbate and the charcoal filtrate when used alone should be so much less effective on spore germination, early mycelial growth, and gametic reproduc-

tion than when used in combination. If either or both extracts were toxic it might be assumed that the phenomenon of antagonism was involved, but each extract is beneficial, which would appear to eliminate that possibility.

The $Z_1$ factor appeared to be concerned primarily in growth and had little effect upon gametic reproduction when used alone with thiamin. On the other hand, the $Z_2$ factor affected favorably both growth and gametic reproduction.

From various observations made during the course of our experiments a suitable proportion of the two factors seemed desirable for optimum results. Too much of fraction Ca (containing the $Z_1$ factor) in proportion to fraction Dr (containing the $Z_2$ factor) appeared, for example, less satisfactory for gametic reproduction than mixtures with smaller amounts of fraction Ca. Of course these conclusions may be modified when purer preparations are available.

It is perhaps premature to discuss the identities of the two factors, but some possibilities may be mentioned.

Factor $Z_1$ has some of the characteristics of biotin. Both are adsorbed by charcoal, from which they may be freed by ammoniacal acetone, and both are thermostable. Biotin has been reported by KÖGL and FRIES (3) to be ineffective for *Phycomyces*, in the presence of thiamin. That would not necessarily eliminate the possibility that factor $Z_1$ is biotin. Our preparations are impure and the Ca fraction might contain traces of the $Z_2$ factor, which would account for the beneficial effect of the Ca fractions when used alone. If $Z_1$ is biotin, it might be assumed that development of *Phycomyces* in a thiamin solution under the conditions we have used is limited by its ability to synthesize factor $Z_2$. When that factor is supplied, its development is limited by its ability to synthesize factor $Z_1$. Under such a hypothesis biotin would be ineffective when used with thiamin and effective when used together with thiamin and factor $Z_2$. Further investigation of the possible identity of factor $Z_1$ and biotin is under way.

The experiments with the anti gray factor concentrate show that it contains considerable $Z_1$ factor. It is uncertain, however, whether the anti gray factor and the $Z_1$ factor are identical. The former is thermostable, resisting autoclaving at 10–15 pounds' pressure for 15 minutes and some hours of boiling temperature; the latter according

to LUNDE and KRINGSTAD (5) is destroyed by 3 hours' boiling in vacuo. On the other hand, MORGAN (6) reports that a yeast eluate heated under a reflux in strongly alkaline solution still retains its activity. The anti gray factor concentrate probably contains several growth substances; we have found, for example, that there is considerable biotin present—as determined by the growth of *Ashbya gossypii* (10).

The possibility that the $Z_1$ factor might be pantothenic acid was considered also, although pantothenic acid (11) has been reported ineffective for *Phycomyces*. But this acid would appear to be more easily destroyed by heat than is the $Z_1$ factor.

Consideration should be given also to the rat growth factor reported by LEPKOVSKY, JUKES, and KRAUSE (4). This factor is adsorbed by charcoal and is resistant to heating, as reported by LUNDE and KRINGSTAD (5).

The $Z_2$ factor does not appear to be m-inositol or vitamin $B_2$, vitamin $B_6$, nicotinic acid, $\beta$ alanine, or adenine.

The possibility that there are more than two factors concerned cannot be neglected. At this stage, however, it would not be advisable to assume more than two.

## Summary

1. Evidence has been presented which confirms previous reports that spore germination, early growth, and gametic reproduction in *Phycomyces* may be stimulated in cultures to which a potato extract has been added. Increasing amounts of potato extract in the culture medium up to a certain amount may increase growth and reproduction; quantities beyond these amounts bring about decrease in growth and zygote formation.

2. Indications are that the potato extract used contains at least two factors which influence development of *Phycomyces*. One factor can be adsorbed on charcoal and is freed from it in the presence of ammoniacal acetone. This factor is designated at present $Z_1$. The other factor is not adsorbed to any great extent on charcoal and following filtration is present in the filtrate. This factor is designated $Z_2$.

3. Either of the factors, when used in the relatively impure state described here, produced some stimulative effect upon development, but a combination of the two stimulated development far more than either used alone.

4. A concentrate of the anti gray factor obtained from Dr. AGNES MORGAN stimulated growth of *Phycomyces* when used alone in solution I but was much more effective when used in combination with the fraction containing the $Z_2$ factor. This may indicate that the anti gray concentrate is especially rich in the $Z_1$ factor.

5. The $Z_1$ factor may be identical with biotin. The properties of the $Z_2$ factor have not as yet been correlated with those of any other known growth factor or vitamin.

NEW YORK BOTANICAL GARDEN
AND
DEPARTMENT OF BOTANY
COLUMBIA UNIVERSITY
UNIVERSITY OF CHICAGO

## LITERATURE CITED

1. BUSTON, H. W., and PRAMANIK, B. N., The accessory factor necessary for the growth of *Nematospora gossypii*. I. The chemical nature of the accessory factor. Biochem. Jour. **35**:1656–1670. 1931.
2. EASTCOTT, E. V., Wildier's bios. The isolation and identification of bios. I. Jour. Physiol. Chem. **32**:1093–1111. 1928.
3. KÖGL, F., and FRIES, N., Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Zeitschr. Physiol. Chem. **269**:23–110. 1937.
4. LEPKOVSKY, S., JUKES, T. J., and KRAUSE, M. E., The multiple nature of the third factor of the vitamin B complex. Jour. Biol. Chem. **115**:557. 1936.
5. LUNDE, G., and KRINGSTAD, H., Über Veranderungen des Pelzes von Ratten durch Mangel an gewissen Faktoren des Vitamin B. Komplex II. Zeitschr. Physiol. Chem. **257**:201. 1939.
6. MORGAN, AGNES F., and SIMMS, HELEN D., Greying of fur and other disturbances in several species due to a vitamin deficiency. Jour. Nutrition **19**:233–250. 1940.
7. ROBBINS, WM. J., Growth substances in agar. Amer. Jour. Bot. **26**:772–778. 1939.
8. ———, Growth substances and gametic reproduction by *Phycomyces*. BOT. GAZ. **101**:428–449. 1939.
9. ———, Effect of extracts of *Phycomyces* upon its development. In press. Amer. Jour. Bot.
10. ROBBINS, WM. J., and SCHMIDT, MARY BARTLEY, Preliminary experiments on biotin. Bull. Torr. Bot. Club **66**:139–150. 1939.
11. SCHOPFER, W. H., Vitamines et facteurs de croissance chez les plantes. Contributions à l'étude quantitative des conditions d'action des facteurs de croissance sur *Phycomyces*. Archiv. Mikrobiologie **6**:510–531. 1935.

# AVENA COLEOPTILE ASSAY OF ETHER EXTRACTS OF APHIDS AND THEIR HOSTS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 513

GEORGE K. K. LINK, VIRGINIA EGGERS, AND JAMES E. MOULTON

## Introduction

This investigation was carried out to determine, by the standard decapitated *Avena* coleoptile test (12), whether ether extracts of aphids are active in this test; and if so, to compare them with ether extracts of the host organ as to kind and magnitude of effect. The study proceeded from the hypothesis that if growth affecting or effecting substances, *i.e.*, auxones (3, 4, 5) play the roles in growth indicated by current theory and experiment, then the growth disturbances noted in many plants parasitized by aphids should be correlated with disturbed auxone relations (dysauxony), and the aphids should play causal roles in these disturbances by affecting the kind, quantity, behavior, and other relations of the relevant auxones, including auxins and auxin inhibitors, if these be involved. Theoretically, the aphid could disturb these relations in part (*a*) by secreting or excreting auxones on to or into the host, and/or (*b*) by withdrawing auxones from the host. Addition, withdrawal, or both, of auxones of the auxin, auxin inhibitor, vitamin, or wound types could disturb the auxone balances characteristic of normal healthy tissues and organs (euauxony).

To date we have tested the extracts of three aphids and their hosts: (1) *Hyalopterus arundinis* on *Phragmites communis;* (2) *Aphis maidis* on *Zea mays;* and (3) *Brevicoryne brassicae* on *Brassica oleracea*. We started with *Hyalopterus arundinis*, even though it does not produce serious local growth disturbances in *Phragmites* leaves, because the large quantities (100 gm.) which then we considered necessary could be collected rather readily. *Aphis maidis* was used because it was available as another grass parasite. *Brevicoryne brassicae* was used because it often incites serious local growth disturb-

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

ances in cabbage and because the aphid and host can readily be kept in a thrifty condition in the greenhouse during the Chicago winter. Because of the latter fact, most of our tests were made with the cabbage aphid and cabbage leaf.

## Investigation

### General methods

Removal of aphids into a glass container by means of a brush of proper stiffness was found the most feasible way to collect them with minimum injury. At the time of collection we also took aphid-free leaf samples of the same rank as the leaves from which aphids were collected.

After weighing, the aphid and leaf sample each was placed in peroxide-free ether (50 cc. per gram fresh material) and extracted in the dark at 0°C. for 22 hours. The ether was then separated from the sample, and from water, evaporated to dryness over a steam-bath, and the residue stored overnight at 0°C. in the dark (**9**). On the next day the residue, here designated extract, was taken up in 2 cc. of melted 1.5 per cent agar, and from this mixture dilutions were prepared by addition of agar so that each new dilution was one-half as concentrated as the previous one. In several experiments, on the day of testing the ether was evaporated to 1–2 cc. and then dropped on to 2 cc. of hot 1.5 per cent agar, from which the initial dilution was made.

The agar dilutions were poured into an $8 \times 10 \times 1.5$ mm. mold. After hardening, the agar cast was cut into twelve equal blocks. These were applied to twice decapitated *Avena* coleoptiles. In each dilution (concentration) tested at least twelve coleoptiles were used; in some tests twenty-four were used. Controls of indoleacetic acid in concentrations of 10, 20, 30, and 40$\gamma$ were run on each day of test, twelve or more coleoptiles per concentration. The curvatures were recorded after 90 minutes. For comparison, the curvature of the 40$\gamma$ indoleacetic acid solution is recorded for each day for testing, although this concentration lies slightly above the proportionality range.

Several preliminary tests were made to determine the kinds of constituents in the extracts. To this end we used VAN OVERBEEK and BONNER's adaptation of the KÖGL, HAAGEN-SMIT and ERXLE-

BEN procedure (**10**) to determine the effect of refluxing with water, 5 per cent HCl, and 0.5 N NaOH upon the activity of different lots of the extracts of *Hyalopterus arundinis* and *Brevicoryne brassicae* and of their hosts. After cooling, each lot was adjusted to slightly acid, and then extracted with ether, the ether separated and evaporated, and the residue taken up in agar and tested.

## Extracts of Hyalopterus arundinis and Phragmites communis

The aphids were covered with honey dew at the time of collection. The plants were vigorous and collections were made on hot July days. Table 1 gives the results of tests with three concentrations of the ether extract of a 1-gm. sample of the aphid.

TABLE 1

Activity of ether extract of Hyalopterus arundinis expressed in average curvature per 12 Avena coleoptiles (degrees)

| Test | Material | Amount extracted (gm.) | Amount tested (gm.)* | | |
|---|---|---|---|---|---|
| | | | 0.5 | 0.25 | 0.125 |
| 20..... | Aphid | 1.0 | 9.8 | 7.1 | 2.6 |
| | Indoleacetic acid 40γ = 19.7° | | | | |

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar.

The extracts of *Hyalopterus arundinis* and of *Phragmites* leaf are active in the coleoptile test (tables 1 and 2). There is a suggestion of proportionality between concentration and activity of the extracts. Lower concentrations of the aphid extract gave better proportionalities than higher ones. Since the lower concentrations of aphid extract and the higher concentrations of leaf extract did not seem to be supramaximal, the greater activity noted for the aphid extract may signify that this differs from the leaf extract as to amount and kind of its effective substances. The extract of the aphid, and possibly of the leaf, each contains a fraction which is active after acid, and another which is active after alkali, hydrolysis (**4**).

## Extracts of Aphis maidis and Zea mays

The plants used for collection of aphids, leaves, and tassels were grown in the greenhouse and had shed their pollen at time of collection. The aphids were rich with honey dew.

TABLE 2

Activity of non-refluxed and refluxed ether extracts of Hyalopterus arundinis and of Phragmites leaf, expressed in average curvature per 12 Avena coleoptiles (degrees)

| Test | Treatment | Material | Amount extracted (gm.) | Amount tested (gm.)* | | | |
|---|---|---|---|---|---|---|---|
| | | | | 5.0 | 2.5 | 1.25 | 0.625 |
| 21 | Not refluxed | Aphid | 10 | 16.0 | 18.0 | 10.0 | 11 |
| 22 | | Leaf | 10 | 4.3 | 1.9 | 1.5 | ..... |
| 21 | $H_2O$ refluxed | Aphid | 10 | 15.0 | 18.0 | 13.0 | ..... |
| 22 | | Leaf | 10 | 3.2 | 0.2 | 2.6 | ..... |
| 21 | 5% HCl refluxed | Aphid | 10 | 6.3 | 15.0 | 13.0 | ..... |
| 22 | | Leaf | 10 | 2.2 | 2.3 | 1.5 | ..... |
| 21 | 0.5 N NaOH refluxed | Aphid | 10 | 9.4 | 3.6 | 5.5 | ..... |
| 22 | | Leaf | 10 | 3.0 | 1.5 | 1.0 | ..... |
| 21 | Indoleacetic acid $40\gamma = 18°$ | | | | | | |
| 22 | Indoleacetic acid $40\gamma = 19.7$ | | | | | | |

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar.

TABLE 3

Activity of ether extracts of Aphis maidis and of leaves and tassels of Zea mays, expressed in average curvature per 12 Avena coleoptiles (degrees)

| Test | Material | Amount extracted (gm.) | Amount tested (gm.)* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 10.0 | 5.0 | 2.5 | 1.25 | 0.125 | 0.062 |
| 27..... | Aphid from leaf | 5 | ..... | ..... | ..... | 7.7 | 9.0 | 4.5 |
| | Leaf | 20 | 40.0 | 33.5 | 26.0 | ..... | ..... | ..... |
| | Aphid from tassel | 5 | ..... | ..... | ..... | 9.2 | 11.7 | 15.0 |
| | Tassel | 20 | 14.2 | 12.7 | 14.0 | ..... | ..... | ..... |
| | Indoleacetic acid $40\gamma = 24.3°$ | | | | | | | |

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar.

The extracts of aphids collected from corn leaves and tassels, as well as the extracts of those organs, were active in the coleoptile

test (table 3). There was no linear proportionality between activity and concentration. While the extract of aphids from the tassel was more active than the extract of the leaf aphid, the extract of the leaf was more potent than the tassel extract for the same concentrations, possibly indicating that these extracts are not identical as to kind and quantity of effective substances. The leaf extract produced pronounced curvatures (40°–26°), whereas the highest curvature noted on the day of testing for indoleacetic acid was 24.3° for a solution containing 40γ per liter. This may indicate that the leaf extract contains a substance or mixture of substances which does not behave like pure indoleacetic acid in the coleoptile test.

### Extracts of Brevicoryne brassicae and of leaves of Brassica oleracea

A great many tests were made with these materials, only a few of which are reported here (tables 4, 5, and 6). For tests 33, 34, and 36 (table 4) the aphids were collected from leaves of various sizes, and comparable aphid-free leaves were used as leaf samples. For tests with small samples (tables 5 and 6) only portions of a single leaf were used. Prior to and at the time of collection, the plants were in a warm room. Collections were made on sunny days from potted plants which had 8–10 leaves each. The aphids were very mealy and of various sizes.

The extracts of the cabbage aphid and cabbage leaf were found active. Each contains a fraction which remains active after acid hydrolysis and one which is active after alkali hydrolysis (table 4). The differences in activity of these fractions of the extracts from 0.62-gm. samples may indicate that the effective substances of the extracts of the aphid and of the leaf differ as to kind and quantity.

Many tests were made in an attempt to find concentrations which would give proportionalities between concentration and activity. The results are so variable from one test to another, and the number of tests possible per day is so limited, that we have not yet succeeded. Variability in leaves and aphids may be due to the fact that even when leaves strictly comparable as to rank and age are used, these leaves and the aphids on them are not the same as to auxone content because they have not experienced the same light and other conditions. If leaves of different ages are used the differences are even greater.

TABLE 4

ACTIVITY OF NON-REFLUXED AND REFLUXED ETHER EXTRACTS OF BREVICORYNE BRASSICAE AND OF LEAVES OF BRASSICA OLERACEA, EXPRESSED IN AVERAGE CURVATURE PER 12 AVENA COLEOPTILES (DEGREES)

| Test | Treatment | Material | Amount extracted (gm.) | Amount tested (gm.)* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 5.0 | 2.5 | 0.25 | 0.62 | 0.31 | 0.15 | 0.07 |
| 33 | Not refluxed | Aphid | 5 | ..... | 4.8 | 9.8 | 10.1 | 16.9 | ..... | ..... |
| 33 | | Leaf | 10 | 1.9 | 0.5 | 2.7 | ..... | ..... | ..... | ..... |
| 34 | $H_2O$ refluxed | Aphid | 5 | ..... | ..... | ..... | 17.1 | 13.7 | 13.0 | 12 |
| 34 | 0.5 N NaOH refluxed | Aphid | 5 | ..... | ..... | ..... | 10.5 | 17.0 | 17.0 | ..... |
| 36 | | Leaf | 10 | ..... | 0.2 | 5.0 | 2.8 | ..... | ..... | ..... |
| 34 | 5% HCl refluxed | Aphid | 5 | ..... | ..... | ..... | 11.2 | 13.8 | 12.0 | ..... |
| 36 | | Leaf | 10 | ..... | 0.1 | 1.4 | 3.7 | ..... | ..... | ..... |

33 Indoleacetic acid 40γ = 16.2°
34 Indoleacetic acid 40γ = 20.0
36 Indoleacetic acid 40γ = 21.0

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar

TABLE 5

ACTIVITY OF ETHER EXTRACTS OF BREVICORYNE BRASSICAE AND OF LEAVES OF BRASSICA OLERACEA, EXPRESSED IN DEGREES CURVATURE OF AVENA COLEOPTILES. DRY RESIDUE KEPT AT 0° C. IN THE DARK AND TESTED AFTER 20 HOURS', 6 DAYS', AND 13 DAYS' STORAGE. AVERAGE CURVATURE PER 12 COLEOPTILES (DEGREES)

| Date (1940) | Test | Material | Amount extracted (gm.) | Amount tested (gm.)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.5 | 0.25 | 0.125 | 0.062 | 0.031 | 0.015 | 0.0075 | 0.0037 |
| Feb. 2 | 45 | Aphid | 0.5 | ..... | 6.6 | 17.3 | 14.2 | 28.4 | ..... | ..... | ..... |
| | | Leaf | 1.0 | 0.4 | 2.0 | 2.3 | 5.8 | ..... | ..... | ..... | ..... |
| Feb. 8 | 45a | Aphid | ..... | ..... | ..... | 23.1 | ..... | 17.3 | 10.6 | 4.0 | ..... |
| | | Leaf | ..... | ..... | 1.0 | ..... | 6.4 | 21.6 | 15.4 | ..... | ..... |
| Feb. 15 | 45b | Aphid | ..... | ..... | ..... | ..... | ..... | 19.8 | 15.1 | 11.4 | 11.9 |
| | | Leaf | ..... | ..... | ..... | ..... | 4.3 | 13.3 | 17.1 | 16.8 | ..... |

45 Indoleacetic acid 40γ = 30.5°
45a Indoleacetic acid 40γ = 34.4
45b Indoleacetic acid 40γ = 26.1

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar.

In an attempt to avoid this difficulty, different portions of the residue of the same extracts were tested on different days (table 5). The ether extracts of the original samples (0.5 gm. aphids and 1 gm. leaf) each was divided into two equal portions. All were reduced to dryness. One each of these portions was used in test 45, while the other portions were stored as dry residues for 5 days. On the fifth day each of the remaining portions were redissolved in ether, which was then divided into two equal portions so as to give again two

TABLE 6

ACTIVITY OF ETHER EXTRACTS OF BREVICORYNE BRASSICAE AND OF LEAVES OF BRASSICA OLERACEA, EXPRESSED IN AVERAGE CURVATURE PER 24 AVENA COLEOPTILES (DEGREES). CONCENTRATED ETHER EXTRACT DROPPED ON HOT AGAR AND TESTED SAME DAY

| TEST | MATERIAL | AMOUNT EXTRACTED (GM.) | AMOUNT USED (GM.)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.125 | 0.0625 | 0.0312 | 0.0156 | 0.0078 | 0.0039 | 0.0019 | 0.0009 |
| 59 | Aphid | 0.25 | 6.4 | 9.8 | 18.5 | 15.7 | ..... | ..... | ..... | ..... |
| | Leaf | 0.25 | 2.6 | 4.8 | 6.3 | 4.4 | ..... | ..... | ..... | ..... |
| 61 | Aphid | 0.25 | 13.4 | ..... | 14.0 | ..... | 3.0 | 2.0 | 0.8 | 0.4 |
| | Leaf | 0.25 | 4.6 | ..... | 8.9 | ..... | 14.9 | 8.9 | 3.8 | 0.7 |
| 59 | Indoleacetic acid 40γ = 16.9° | | | | | | | | | |
| 61 | Indoleacetic acid 40γ = 18.1 (average per 36 coleoptiles) | | | | | | | | | |

* No. of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5% agar.

portions of aphid extract and two portions of leaf extract. Each was evaporated to dryness and one each of these portions was tested the next day (test 46), while the others were kept for 7 more days and then tested (test 47). Because the values obtained with the first and second tests appeared supramaximal for both aphid and leaf extracts, progressively higher dilutions were used in tests 46 and 47. In addition, some of the dilutions of the preceding tests were used as controls. The extracts were highly active even after 13 days of storage as dry residues. On the thirteenth day, dilutions whose residue contents per cc. of agar represented the extracts of 4.8 mg. of fresh leaf and aphid materials respectively gave pronounced curvatures. Even at these low concentrations no good proportionalities were obtained between activity and concentration.

Because it seemed possible that some of the irregularities in results might be due to the method of dilution used and to changes due to evaporation to dryness, experiments were conducted in which the extracts were not reduced to dryness (table 6). In these tests the ether extracts were evaporated to 1–2 cc. and then dropped on to 2 cc. of hot 1.5 per cent agar. From this mixture 1 cc. was removed; to the remainder, 1 cc. of agar was added to give the second dilution. This was repeated until the desired dilutions (concentrations) had been obtained.

Even though very low concentrations were used in test 59 (0.125–0.0156 gm. of material), no good proportionalities were obtained between concentration and activity. For this reason another collection of leaf and aphids was made from the plants used for test 59. A week intervened between collections. Two of the concentrations used in test 59 were included in test 61 as controls, and in addition four other dilutions were prepared. In order to cover a wide range, the first two concentrations were prepared each by fourfold, and the other four each by twofold, dilutions.

Table 6 indicates that the samples of tests 59 and 61 were not identical as to the active substances under test, showing that comparative quantitative tests are very difficult, if not impossible, under the methods in use at present. The results, unlike those of table 5, also show that the higher dilutions gave better proportionalities between concentration and activity. The best values were obtained with extract equivalents between 1.9 and 30 mg. of fresh material per 1 cc. of agar. The leaf extract was the more active, test 61 indicating that the relatively low values in the higher concentrations of leaf extracts of other tests may have been due to supramaximal concentrations.

In control tests of the ether used for extraction, and of the ether distilled off from these extracts, it was found that the distillate of both cabbage leaf and cabbage aphid extracts contained substances active in the coleoptile test. The distillates of the extracts of test 61 were concentrated to 1–2 cc. and then dropped on to 1 cc. of hot agar. The distillates of the leaf extract and of the aphid extract produced curvatures of 14° and 4.4°, respectively. These findings indicate that the ether extracts of cabbage aphid and leaf contain highly volatile constituents which are effective in the coleoptile test and that the

extracts should not be evaporated to dryness. They indicate also that the leaf and aphid extracts are more active than indicated in table 6, and that the differences between them in favor of the leaf extract are even greater than indicated.

## Discussion

The data definitely show that ether extracts of the three species of aphids tested, and of the plant organs on which they feed, are active in the *Avena* coleoptile test. Curvatures were produced by the extracts of 0.25-gm. samples of cabbage leaf and of cabbage aphid, in dilutions equivalent respectively to 2 mg. and 4 mg. of fresh material per cc. of agar.

The data also show that the extracts of *Hyalopterus arundinis* and of *Brevicoryne brassicae*, as well as the extracts of leaves on which they feed, each contains a fraction resistant to alkali hydrolysis and another fraction resistant to acid hydrolysis.

The same concentrations of aphid and leaf extracts are not equally active. This may indicate that the effective substances in the extracts differ as to kind and quantity. The *Phragmites* aphid extract was more active than the *Phragmites* leaf extract. The data of the corn aphid and corn leaf tests are too meager and the difference in amount of material used is too great to warrant conclusions. The cabbage aphid extract in high concentrations was much more active than the leaf extract in the same concentrations. In the lower concentrations this relationship did not always hold. We had hoped to obtain comparative quantitative data for the aphid and the leaf extracts. The data, however, are characterized by lack of linear proportionality between activity and concentration of the extract in the agar dilutions. This was most marked in the cabbage aphid and cabbage leaf extracts, and applies even to dilutions in which 1 cc. of agar contained the extracts from 2 to 3 mg. of fresh aphid or cabbage leaf.

While for the lower dilutions these discrepancies may be due to supramaximal amounts of active substances, it does not seem likely that supramaximal quantities alone are involved when dilutions of extracts equivalent to 2–10 mg. of fresh material are used. Similar discrepancies were noted by LINK and EGGERS (4). The data indicate that a wide range of dilutions (concentrations) must be tested

before it is permissible to select any curvature value which falls within the proportionality range of indoleacetic acid as an index of the amount of active substance contained in the sample under analysis.

Possibly the discrepancies in proportionality between concentration and activity are in part due to the presence of active substances which affect the coleoptile unequally, both in kind and intensity. The results of a considerable body of investigators indicate that the growth effects obtained in the coleoptile test with ether extracts of plant materials—and interpreted as auxin effects—may be the net effects of mixtures of substances, some of which favor and some of which prevent cell elongation. The work of GOODWIN (1), STEWART, BERGREN, and REDEMAN (8), STEWART (7), LARSEN (2), VOSS (11), and SNOW (6) definitely indicates a multiplicity of substances with inhibitory effects, and LARSEN'S work shows that several classes of these substances may occur in the extract of one plant organ.

These uncertainties and lack of knowledge as to the constituents of ether extracts affective and effective in the coleoptile test render premature any conclusions as to the kind of auxins present in fractions of extracts active after acid or alkali hydrolysis (4).

Perhaps better proportionalities might have been obtained if more coleoptiles had been tested. Even with pure indoleacetic acid solution, some coleoptiles on the same day—and whole sets of coleoptiles from day to day—show curvatures more than twice that obtained in others treated with the same concentration. Reasonably good proportionalities are obtained for activity and concentration of indoleacetic acid only by averaging the results of many tests.

Possibly the discrepancies are due to improper or irregular treatment of the effective substances in the processes of extraction, concentration, or dilution with agar. Better proportionalities were obtained when the ether extracts of cabbage leaf and aphids were not evaporated to dryness. The fact that active substances were recovered from the distillates of these ether extracts indicates that the active portions contain highly volatile and possibly unstable substances. This observation is partially offset by another to the effect that dry residues, redissolved in ether and reduced to dryness a second time, were highly active after 13 days' storage.

So far it has not been proved that any of the methods of extrac-

tion in use quantitatively remove the active substances under test. Another great difficulty in the way of comparable quantitative results is the limited number of tests possible on one day and the variability from day to day of the leaf and aphid material.

In the light of these considerations it appears premature to draw conclusions as to the significance of differences in magnitude of activity for equal concentrations of different samples in terms of amounts and kinds of effective substances.

Until these problems are solved, no answer can be given to the question whether an aphid whose extract is effective in the coleoptile test derives its effective substances from host leaf cells from which it sucks its food or whether, at least in part, it itself (and/or some organism within its digestive tract) makes some or all of these substances from the materials obtained from the host. Hence no answer can be formulated to the question whether an aphid incites auxone disturbances and whether these play roles in the growth disturbances associated with aphid parasitism.

## Summary

1. Ether extracts of the aphids *Hyalopterus arundinis*, *Aphis maidis*, and *Brevicoryne brassicae* and of their respective hosts, *Phragmites communis*, *Zea mays*, and *Brassica oleracea*, consistently produced negative curvatures in the *Avena* coleoptile test. Extracts of the cabbage aphid and of the cabbage leaf were active in amazingly high dilutions.

2. Residues of equal samples of aphid and host extracts were not equally active. The extract of *Hyalopterus arundinis* was more active than the *Phragmites* leaf extract. The extract of the mealy cabbage aphid in higher concentrations was more active than the extract of the cabbage leaf; in lower concentrations this was not always the case.

3. Extracts of *Hyalopterus arundinis* and of *Brevicoryne brassicae* and of the hosts *Phragmites communis* and *Brassica oleracea* each contains a fraction which is active after acid, and another which is active after alkali, hydrolysis.

4. The results, especially in the tests with higher concentrations of extracts of cabbage aphid and cabbage leaf, are characterized by

lack of proportionality between amount of sample extracted and activity of the residue of the ether extract in agar.

5. Distillates from the ether extracts of the cabbage leaf and cabbage aphid contain substances active in the coleoptile test.

6. Dry residues of the extracts of the cabbage aphid and leaf were active after six days' storage and also after thirteen days' storage in the dark at 0° C., following a second solution in ether and evaporation to dryness. Better proportionalities between concentration and activity of the extracts were obtained when the extracts were not evaporated to dryness.

UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

## LITERATURE CITED

1. GOODWIN, R. H., Evidence for the presence in certain ether extracts of substances partially masking the activity of auxin. Amer. Jour. Bot. **26**:130. 1939.
2. LARSEN, PAUL, Über Hemmung des Streckenwachstums durch natürlich vorkommende, Ätherlösliche Stoffe. Planta **30**:160–167. 1939.
3. LINK, G. K. K., WILCOX, H. W., and LINK, A. DE S., Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts, β indoleacetic acid, and wounding. BOT. GAZ. **98**:816–867. 1937.
4. LINK, G. K. K., and EGGERS, VIRGINIA, Aphids, auxones, pseudogalls, and galls. Amer. Jour. Bot. **25**:155. 1938.
5. LINK, G. K. K., and EGGERS, VIRGINIA, *Avena* coleoptile assay of ether extracts of nodules and roots of bean, soybean, and pea. BOT. GAZ. **101**: 650–657. 1940.
6. SNOW, R., An inhibitor of growth extracted from pea leaves. Nature **144**: 906. 1939.
7. STEWART, W. S., A plant growth inhibitor and plant growth inhibition. BOT. GAZ. **101**:91–108. 1939.
8. STEWART, W. S., BERGREN, W., and REDEMAN, E. E., A plant growth inhibitor. Science **89**:185. 1939.
9. VAN OVERBEEK, J., A simplified method for auxin extraction. Proc. Nat. Sci. **24**:42–46. 1938.
10. VAN OVERBEEK, J., and BONNER, J., Auxin in isolated roots growing in vitro. Proc. Nat. Acad. Sci. **24**:200–264. 1938.
11. VOSS, H., Nachweis des inaktiven Wuchsstoffes, eines Wuchsstoff antagonisten und deren Wachstumsregulatorische Bedeutung. Planta **30**:252–285. 1939.
12. WENT, F. W., and THIMANN, K. V., Phytohormones. p. 283. Macmillan, New York. 1937.

# AUXIN IN MARINE PLANTS. II[1]

J. VAN OVERBEEK

The presence of auxin has been reported in the brown alga *Macrocystis*, the green alga *Bryopsis*, and also in *Elodea* (**9**). Previous workers had demonstrated it in *Valonia* (**5**) and *Fucus* (**2**). During the summer of 1939, investigations were carried out at the Oceanographic Laboratories of the University of Washington at Friday Harbor, Washington, in order to study the distribution of auxin among marine plants. Since the waters near the San Juan Islands provide an ideal habitat for the giant kelp *Nereocystis*, additional investigations were made on this plant to learn its auxin distribution.

## Method

The ether extraction technique (**7, 8, 9**) for plant hormones of the auxin type was used. The auxin was analyzed by means of the standard *Avena* test (**11**), improved according to Schneider and Went (**3**). The darkroom was controlled at 24°–25° C. and 80–85 per cent relative humidity. The response to auxin of the *Avena* test plants at Friday Harbor proved relatively high (table 1) and varied little from day to day, in contrast to pronounced variations in other localities (**11**). At Friday Harbor there also appeared to be a satisfactory direct proportionality between the auxin concentration and the curvature of the test plants. As shown in table 1, this proportionality is often lacking at Pasadena (**6**), which somewhat complicates the evaluation of the test (**10**).

The auxin concentrations in the extracted material were expressed in terms of the indoleacetic acid controls as previously (**7, 8, 9, 10**). If an extract of $W$ grams of fresh plant material is taken up in $V_a$ cc. of agar and blocks of the latter give a curvature of $C$ in the *Avena* test, then the auxin concentration in the plant material $= \dfrac{C \times I_1^{\circ} \times V_a}{W}$ $\gamma$ indoleacetic acid equivalents per kilogram fresh weight. $I_1^{\circ}$ is the indoleacetic acid concentration ($\gamma$/l.)

[1] Contribution no. 91, Oceanographic Laboratories, University of Washington, Seattle.

causing $C$ to increase by one degree; it is the factor that transforms curvature into concentration. In tables 2 and 3, $W$, $V_a$, and $C$ are given in addition to the auxin concentration in order to facilitate repetition and extension of the experiment by later workers. The value of $I_{1^\circ}$ can readily be obtained from table 1. In practice, a curve

TABLE 1

RESPONSE OF AVENA TEST PLANTS TO INDOLEACETIC ACID DARKROOM CONDITIONS: TEMPERATURE 24°–25° C.; HUMIDITY 80–85%. AVERAGE OF TWELVE PLANTS IN FRIDAY HARBOR SERIES AND OF TWENTY-FOUR PLANTS IN PASADENA SERIES

| DATE (1939) | 25γ/l. | 50γ/l. |
|---|---|---|
| | Friday Harbor | |
| July 22 | 7.5 | 15.8 |
| | 8.6 | 15.3 |
| July 27 | 7.8 | 18.5 |
| Aug. 2 | 8.0 | 16.2 |
| Aug. 7 | 8.6 | ..... |
| Aug. 11 | 9.4* | ..... |
| Aug. 13 | 7.4* | ..... |
| Aug. 14 | 7.2* | ..... |
| | 8.3* | ..... |
| Aug. 18 | 7.4* | ..... |
| Average | 8.0±0.22 (2.7%) | ..... |
| | Pasadena | |
| Sept. 13 | 7.5±0.51 | 13.1±0.62 |
| Sept. 15 | 2.4±0.20 | 9.2±0.62 |
| Sept. 19 | 9.9±0.52 | 9.2±0.85 |

* New solution.

is made in which the known control concentrations of indoleacetic acid are plotted on the abscissa against the curvature on the ordinate. Using this graph, for any given value of $C$ (below the maximum angle) on the ordinate, the corresponding value of $(C \times I_{1^\circ})$ may be found on the abscissa.

## REPRODUCIBILITY

If an auxin analysis has been made of a certain marine alga and a figure found for its auxin content, it may be asked how significant

this figure is. This question was answered by a series of experiments, summarized in table 2. During the months of July and August, sections about 4 inches long located near the middle of fronds of *Nereocystis* were extracted. The plants were collected from a single locality, Reid Rock in the San Juan Channel, at slack tide. Table 2 shows that the auxin content, with but one exception, never varied

TABLE 2

CONCENTRATIONS OF AUXIN IN MIDDLE PART OF FRONDS OF NEREOCYSTIS
DATA SHOW REPRODUCIBILITY OF EXTRACTION TECHNIQUE

| DATE (1939) | EXTRACTION TIME (DAYS) | WEIGHT OF MATERIAL (GM.) | VOLUME OF AGAR (CC.) | CURVATURE IN AVENA TEST (DEGREES) | AUXIN CONCENTRATION (γ/KG. INDOLEACETIC ACID EQUIVALENTS) |
|---|---|---|---|---|---|
| July 14 | 2 | 57 | 0.4 | 10.8 | 0.24 |
| July 15 | 3 | 80 | 0.6 | 9.0<br>10.8 | 0.23 |
| July 20 | 7 | 116 | 0.9 | 8.8<br>11.7 | 0.21<br>0.28 |
| July 22 | 2 | 100 | 0.7 | 19.0 (max.)<br>19.6 | ..... |
| July 22 | 2 | 100 | 1.4 | 17.0 | 0.73 |
| July 27 | 2 | 100 | 1.5<br>3.0 | 5.7<br>3.9 | 0.24<br>0.33 |
| Aug. 11 | 2 | 100 | 1.0<br>2.0 | 11.1<br>5.5 | 0.34<br>0.36 |
| Aug. 12 | 3 | 100 | 1.0<br>2.0 | 4.8<br>2.5 | 0.14<br>0.16 |
| Aug. 13 | 4 | 100 | 1.0 | 6.2<br>6.3 | 0.20 |
| Aug. 16 | 3 | 140 | 1.0 | 12.0 | 0.26 |
| Average | ..... | ..... | ..... | ..... | $0.29 \pm 0.042$ (14.5%) |

considerably from 0.29 γ/kg. The standard deviation was about 15 per cent. This seems satisfactory if the fact that the experimental material was grown under natural conditions is taken into account.

## DISTRIBUTION OF AUXIN AMONG MARINE PLANTS

Table 3 summarizes the auxin analyses of a number of marine plants and one salt-marsh plant. It is evident from these data that auxin is present in practically all the plants investigated, and one seems justified in generalizing that auxin is present in all marine plants. The few negative auxin tests can be traced back to technicalities rather than to a basic lack of auxin.

In *Phaeophyta* the auxin concentration varied roughly between 0.05 and 0.5 γ/kg. indoleacetic acid equivalents. The lowest content

TABLE 3

SUMMARY OF AUXIN ANALYSES OF MARINE PLANTS FOUND NEAR SAN JUAN ISLANDS

| NAME | PART ANALYZED | DATE (1939) | EXTRACTION TIME (DAYS) | WEIGHT (GM.) | AGAR VOLUME (CC.) | CURVATURE IN AVENA TEST | AUXIN CONCENTRATION (γ/KG. INDOLEACETIC ACID EQUIVALENTS) |
|---|---|---|---|---|---|---|---|
| Brown algae | | | | | | | |
| Agrum sp. | Old fronds | July 25 | 2 | 65 | 0.3 | 8.3 | 0.12 |
| Alaria valida | Sporophylls | July 16 | 2 | 38 | 0.3 | Agar failed to harden | |
| Costeria costata | Old hapteres | July 25 | 2 | 28 | 0.3 | 9.4 | 0.30 |
| Costeria costata | Old fronds | July 25 | ..... | 75 | 0.3 | +0.4 | ..... |
| Cystophyllum geminatum | Young thalli | July 16 | 4 | 30 | 0.3 | 11.7 | 0.37 |
| Desmarestia aculeata | Thallus | July 20 | 2 | 50 | 0.6 | 1.0 | 0.04 |
| Desmarestia aculeata | Thallus | July 25 | 2 | 35 | 0.3 | 5.6 | 0.15 |
| Desmarestia munda | Lateral blades | July 25 | 2 | 78 | 0.3 | 0.4 | ..... |
| Desmarestia munda | Thallus | July 20 | 2 | 52 | 0.6 | 2.2 | 0.08 |
| Egregia menziesii | Young thalli | July 16 | 4 | 55 | 0.5 | 6.9 | 0.20 |
| Fucus evanescens | Tips of vegetative thallus | July 15 | 2 | 25 | 0.3 | 14.2 | 0.54 |
| Fucus evanescens | Tips of vegetative thallus | Aug. 8 | 1 | 25 | 0.5 | 1.1 | 0.08* |
| Fucus evanescens | Base of vegetative thallus | Aug. 8 | 1 | 50 | 0.5 | 16.7 | 0.53* |
| Fucus evanescens | Bladders of fertile thallus | July 15 | 2 | 50 | 0.3 | 17.2 (max.) | ..... |
| Fucus evanescens | Bladders of fertile thallus | Aug. 8 | 1 | 25 | 0.5 | 8.0 | 0.50* |
| Fucus evanescens | Base of fertile thallus | Aug. 8 | 1 | 50 | 0.5 | 7.0 | 0.22* |
| Hedophyllum sessile | Base and middle of frond | July 16 | 4 | 82 | 0.5 | 8.5 | 0.15 |
| Laminaria sp. | Frond | July 15 | 2 | 23 | 0.3 | 0.2 | ..... |
| Pleurophycus gardneri | Basal part of frond | July 16 | 4 | 67 | 0.5 | 15.2 | 0.37 |
| Red algae | | | | | | | |
| Iridaea heterocarpa | Entire plant | July 16 | 4 | 57 | 0.5 | 4.4 | 0.12 |
| Rhodymenia sp. | Fronds with tetraspores | July 18 | 2 | 49 | 0.3 | 12.0 | 0.23 |
| Rhodymenia sp. | Fronds with proliferations | July 18 | 2 | 41 | 0.3 | 0.8 | 0.02 |
| Diatoms | | | | | | | |
| Melosira and Biddulphia | Mixed colonies | July 24 | 2 | 4 | 0.3 | 9.3 | 2.20 |
| Higher plants | | | | | | | |
| Phyllospadix scouleri | Fruiting parts | July 16 | 4 | 15 | 0.3 | 12.1 | 0.76 |
| Phyllospadix scouleri | Fruiting parts | Aug. 15 | 2 | 40 | 1.0 | {13.7 12.0 | 1.0* |
| Phyllospadix scouleri | Middle part of leaves | July 16 | 4 | 18 | 0.3 | 5.3 | 0.28 |
| Phyllospadix scouleri | Middle part of leaves | Aug. 15 | 2 | 70 | 1.0 | {5.6 4.1 | 0.21* |
| Zostera marina | Flowering parts | July 15 | 2 | 28 | 0.3 | 13.1 | 0.45 |
| Zostera marina | Flowering parts | July 22 | 4 | 100 | 3.6 | 8.1 | 0.47 |
| Salicornia ambigua | Fertile tips | July 24 | 2 | 30 | 0.3 | 9.3 | 0.30† |
| Salicornia ambigua | Vegetative tips | July 24 | 2 | 30 | 0.3 | 7.4 | 0.23† |

* Analysis by Miss FRANCES BJORKMAN.

† Analysis by Dr. G. B. RIGG.

was found in *Desmarestia* and the highest in *Fucus*. *Desmarestia* is of physiological interest because of the high acidity of its cell sap,

which is reported to have a pH of about 1 (12). The auxin concentrations found in the brown algae near Friday Harbor were of the same order of magnitude as those found in *Macrocystis* near Newport Bay in California (9).

Plants of *Rhodophyta* were analyzed, for the first time so far as the writer is aware. Table 3 shows that their auxin content is approximately the same as that of the brown algae. *Rhodymenia* thalli occasionally have peculiar outgrowths all over their surface. Some of these thalli were analyzed and found to contain less auxin than those bearing tetraspores. This, however, does not necessarily indicate a relation between auxin content and formation of these proliferations, because the thalli with proliferations looked as though they were considerably older than the ones bearing tetraspores.

The Bacillariophycae were also analyzed. On pilings in rather stagnant water large colonies which were a mixture of *Melosira* and *Biddulphia* were found. The excess water was carefully removed by blotting between filter paper, and the diatoms were weighed and extracted. The auxin concentration, on the basis of fresh weight, was higher than in the other algae investigated. The presence of auxin in rather high concentrations in diatoms again stresses the point that for a correct analysis of the larger algae they must be free from overgrowth.

Two representatives of the Naiadaceae were also analyzed. The fruiting parts of *Phyllospadix* contained considerably more auxin than the leaves, which is also commonly experienced in land plants (11). Because *Zostera* usually grows in much more protected waters than *Phyllospadix*, it was found impossible to collect leaves of it that were free from overgrowth, and such leaves were therefore not analyzed. The spadix of *Zostera*, however, is inclosed in a spathe and was therefore free from contamination by other organisms.

Also an analysis of the young tips of *Salicornia ambigua* was made. The tips of fertile plants were found to have a higher auxin content than tips of sterile plants.

## Distribution of auxin in Nereocystis

*Nereocystis*, like *Macrocystis*, belongs to the order of the Laminariales. It is an annual, in contrast to *Macrocystis*, which is perennial. The unbranched tips, which may reach a length of over 20 meters,

terminate in a large pneumatocyst.[2] Above the bladder are numerous short dichotomously forked branches (4), each terminating in a blade that may reach a length of 7 meters. Sori develop on the blades as patches approximately 30–50 cm. long and occupying about three-fourths of the width of the blade. When the sori are ripe, a white line appears around them and they fall out, leaving long slips of the margin of the blade. The oldest sori are located near the apex of the blades.

For the auxin analysis, plants collected at Reid Rock were used exclusively. These plants were large and free from overgrowth, in

TABLE 4

DISTRIBUTION OF AUXIN IN NEREOCYSTIS. FRONDS WERE 5–7 M. LONG WITH STIPE OF ABOUT 13 M.

| SECTION | AUXIN CONCENTRATION | | | |
|---|---|---|---|---|
| | JULY 22 | | JULY 27 | |
| | γ/KG. | PERCENTAGE | γ/KG. | PERCENTAGE |
| Upper part of blades | 0.29 | 17.4 | 0.12 | 18.9 |
| Middle part of blades | 0.73 | 44.0 | 0.24<br>0.33 | 45.0 |
| Basal part of blades | 0.36 | 21.5 | 0.14 | 22.0 |
| Petiole-like part of frond | 0.25 | 15.0 | 0.02 | 3.1 |
| Bladder | 0.024 | 1.4 | 0.017 | 2.7 |
| Upper part of stipe | 0.0 | 0.0 | 0.009 | 1.4 |
| Middle part of stipe | 0.021 | 1.3 | 0.010 | 1.6 |
| Basal (solid) part of stipe | 0.0 | 0.0 | 0.031 | 4.9 |

contrast to the smaller plants which were less exposed to strong tidal currents. Sections about 10 cm. long, sampled at random from the many blades, were taken from the apical, middle, and basal parts of the blades. The short forked branches between the bladder and the base of the fronds were all used for extraction. Also the entire bladder was extracted. From the stipe relatively large parts were taken from the apex (region below the bladder), middle, and the solid base. No hapteres were analyzed since they could not be collected. Table 4 summarizes the analyses of two experiments. It

[2] The gas in this bladder and in the hollow part of the stipe is of peculiar composition. According to LANGDON (1) it contains on the average 4 per cent of CO. Occasionally concentrations as high as 12 per cent CO were found. Little $CO_2$ was found and 18 per cent $O_2$. The gas pressure is below the atmospheric pressure.

shows that the blades have an auxin concentration considerably higher than that of the stipe. A similar relation was found in *Macrocystis* (9). The high auxin content of the blades probably indicates that auxin is produced there. The highest auxin concentration was found in the middle part; the apex and base contained less. The forked branches at the apex of the bladder contained 0.25 γ/kg. in one experiment and only 0.02 γ/kg. in another. This is probably correlated with the higher auxin content of the blades in the first experiment. The auxin probably moves downward from the blades into the branches, the bladder, and the stipe. The negative

TABLE 5

AUXIN CONCENTRATIONS OF SORAL PATCHES AND ADJACENT TISSUE OF NEREOCYSTIS

| DATE (1939) | SECTION | AUXIN CONCENTRATION (γ/KG.) |
|---|---|---|
| July 22 | Youngest sori of frond | 0.16 |
| | | 0.13 |
| | Part of frond just below youngest sori | 0.46 |
| | | 0.46 |
| July 27 | Oldest sori, ready to fall | 0.68 |
| | Margin of blade around oldest sori | 0.48 |
| | | 0.43 |
| | Basal part of frond, just below youngest sori | 0.15 |

results of the analysis of the upper and basal part of the stipe in the first experiment were due to an insufficient amount of material used for the extraction. In the second experiment larger amounts were used and auxin was found to be present.

Table 5 shows the results of an attempt to compare the auxin content of the sori with that of the rest of the blades. Although these experiments can be regarded only as preliminary, they seem to indicate that the mature sori have a higher auxin content than the margin of the blades around them. The youngest sori contained less auxin than the basal part of the blade just below them, on which no sori could be detected.

## Summary

1. Auxin was found in practically all the marine plants analyzed during the summer of 1939. It occurred in brown and red

algae in concentrations ranging from approximately 0.05 γ/kg. to 0.5 γ/kg. indoleacetic acid equivalents (table 3), which is less than in many higher land plants. It also was found in diatoms and in *Phyllospadix* and *Zostera*. The fertile parts of *Phyllospadix* contained considerably more auxin than the leaves (table 3).

2. The blades of *Nereocystis* were found to have an auxin concentration at least ten times higher than that of the stipe, indicating that auxin is produced in the blades. The middle part of the blades had the highest auxin content (table 4).

3. The sensitivity of the *Avena* test plants to standard indoleacetic acid solution was very uniform from day to day at Friday Harbor (table 1).

The writer is indebted to Dr. THOMAS G. THOMPSON, Dr. GEORGE B. RIGG, and Dr. LYMAN D. PHIFER for the hospitality of their laboratories, and to Miss FRANCES BJORKMAN, who skillfully assisted with the analyses.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

## LITERATURE CITED

1. LANGDON, S. C., Carbon monoxide in the pneumatocyst of *Nereocystis*. Puget Sound Mar. Sta. Publ. **1**:237–246. 1916.
2. OLSON, R. A., and DU BUY, H. G., The role of growth substance in the polarity and morphogenesis of *Fucus*. Amer. Jour. Bot. **24**:611–615. 1937.
3. SCHNEIDER, C. L., and WENT, F. W., A photokymograph for the analysis of the *Avena* test. BOT. GAZ. **99**:470–496. 1938.
4. SMITH, G. M., Cryptogamic botany. I. McGraw-Hill, New York. 1938.
5. VAN DER WEY, H. G., On the occurrence of growth substance in marine algae. Proc. Kon. Akad. Wetensch. Amsterdam **36**:759–760. 1933.
6. VAN OVERBEEK, J., Growth substance curvatures of *Avena* in light and dark. Jour. Gen. Physiol. **20**:283–309. 1936.
7. ———, A simplified method for auxin extraction. Proc. Nat. Acad. Sci. **24**:42–46. 1938.
8. ———, Auxin distribution in seedlings and its bearing on the problem of bud inhibition. BOT. GAZ. **100**:133–165. 1938.
9. ———, Auxin in marine algae. Plant Physiol. **15**:291–299. 1940.
10. VAN OVERBEEK, J., and BONNER, JAMES, Auxin in isolated roots growing in vitro. Proc. Nat. Acad. Sci. **24**:260–264. 1938.
11. WENT, F. W., and THIMANN, K. V., Phytohormones. Macmillan, New York. 1937.
12. WIRTH, H. E., and RIGG, G. B., The acidity of the juice of *Desmarestia*. Amer. Jour. Bot. **24**:68–70. 1937.

# MORPHOLOGICAL STUDY OF EXORMOTHECA TUBERIFERA

SULTAN AHMAD

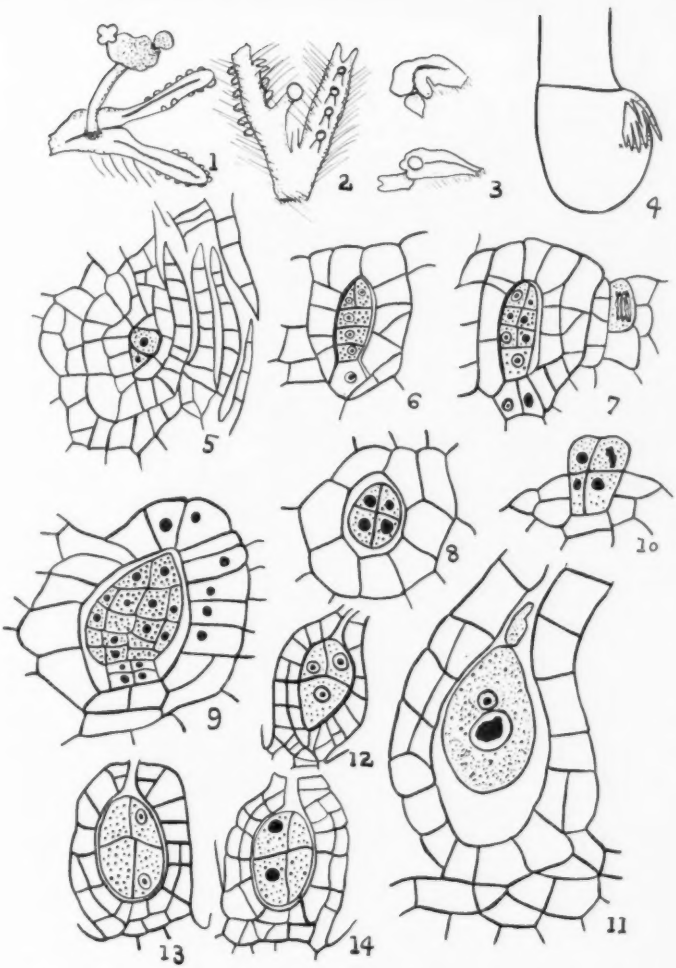
(WITH TWENTY-FIVE FIGURES)

*Exormotheca tuberifera* Kash. was described by KASHYAP (1) in 1914. Later a detailed investigation of the species was undertaken at his suggestion, as this only Indian species presented several points of interest. The material was collected by the writer at Simla in August, 1932, but as the plants were rather advanced in age the earlier stages of development could not be studied in detail. The material was fixed in formalin-acetic-alcohol and sections were cut 4–7 $\mu$ thick. They were stained with iron-alum-haematoxylin.

## Gametophyte

The plant consists of a once or twice dichotomously branched thallus (fig. 1). The upper surface is green, with a deep, narrow, median groove. There is a dorsal layer of air chambers which open on the outside by pores which are raised slightly above the surface. These pores are surrounded by a variable number of cells similar to the epidermal cells. The chambers are full of filaments, the terminal cells of which are elongated, with very few chloroplasts. The midrib projects strongly downward into the soil. The ventral surface bears two rows of purple lunate scales which are unappendaged and without mucilage cells.

The plant bears tubers which are formed either at the apex or on the ventral surface of the thallus (fig. 2). Each tuber consists of a short cylindrical stalk about 0.5 mm. in length and a spherical body about 0.4 mm. in diameter. The wall of each tuber is formed of one or two layers of cells which are almost without contents. Their walls are corky and become yellow on treatment with iodine but do not swell or turn blue on subsequent treatment with strong sulphuric acid. Some cells of the outer covering develop into long rhizoids which remain in continuity with the cells from which they arise. The wall of the rhizoids and basal cells of the stalk differ from those of the corky layer. On treatment with iodine and strong sulphuric acid they turn blue, but do not swell appreciably.




FIGS. 1–14.—Fig. 1, plant showing female receptacle. Fig. 2, ventral side showing tubers. Fig. 3, two plants showing continuation of growth after formation of receptacle. Fig. 4, longitudinal section of tuber. Fig. 5, portion magnified to show apical cell. Fig. 6, division of outer cell of young antheridium forming filament of four cells. Fig. 7, antheridium initial dividing and showing formation of vertical walls. Fig. 8, young antheridium in transverse section. Fig. 9, same showing formation of periclinal walls. Fig. 10, young archegonium showing formation of vertical walls. Fig. 11, male nucleus lying near female nucleus. Fig. 12, young sporophyte showing epibasal cell divided by wall. Fig. 13, quadrant stage. Fig. 14, octant stage.

Within the protective covering the cells lie closely packed and are filled with reserve food material. The cell walls of this portion of the tuber are thin and consist of unchanged cellulose. Each cell contains a nucleus and many granules imbedded in the protoplasm.

The cells in the vicinity of the growing point contain proteid granules which stain brown with iodine. The rest of the tissue contains considerable quantities of starch, assuming a brilliant blue color on treatment with iodine.

At one point on the tuber there is a small depression in which is the apical cell, which is covered with numerous minute purple scales (figs. 4, 5).

The plant is monoecious. The antheridia are produced in the middorsal groove in the tissue of the thallus behind the female receptacle; or, if fertilization fails to occur, the two lobes of the thallus begin to form antheridia in the median groove.

The antheridium initial arises a few segments behind the apical cell. It is divided by a transverse wall into a lower stalk cell and an upper antheridium proper (fig. 7). The upper cell divides by transverse walls, resulting in a row of four cells (fig. 6). Vertical walls are later formed in two planes at right angles to each other (figs. 7, 8). Periclinal walls next occur, separating the inner spermatogenous cells from the outer sterile wall cells (fig. 9). The basal cell, after some divisions, forms a short antheridial stalk. When mature, each antheridium fills the cavity of the chamber in which it lies; leaving no space between its large hyaline wall cells and the wall of the chamber. Each chamber opens above by a papilla which is colorless at first but assumes a red color when the antheridium has discharged its contents.

The archegonia are borne in a receptacle which arises as a rounded outgrowth from a pit situated in the angle between the two vegetative shoots. As in most of the higher Marchantiales, the formation of the receptacle terminates the continued dichotomy of the growing apex; but two curious plants were seen where further growth of the thallus was resumed by an apical innovation (fig. 3). This recalls the condition prevalent in *Preissia* and *Stephensoniella*. The receptacle bears two involucres although occasionally only one may be found. Each involucre contains one to four archegonia but generally only one sporogonium matures. If the archegonia are not ferti-

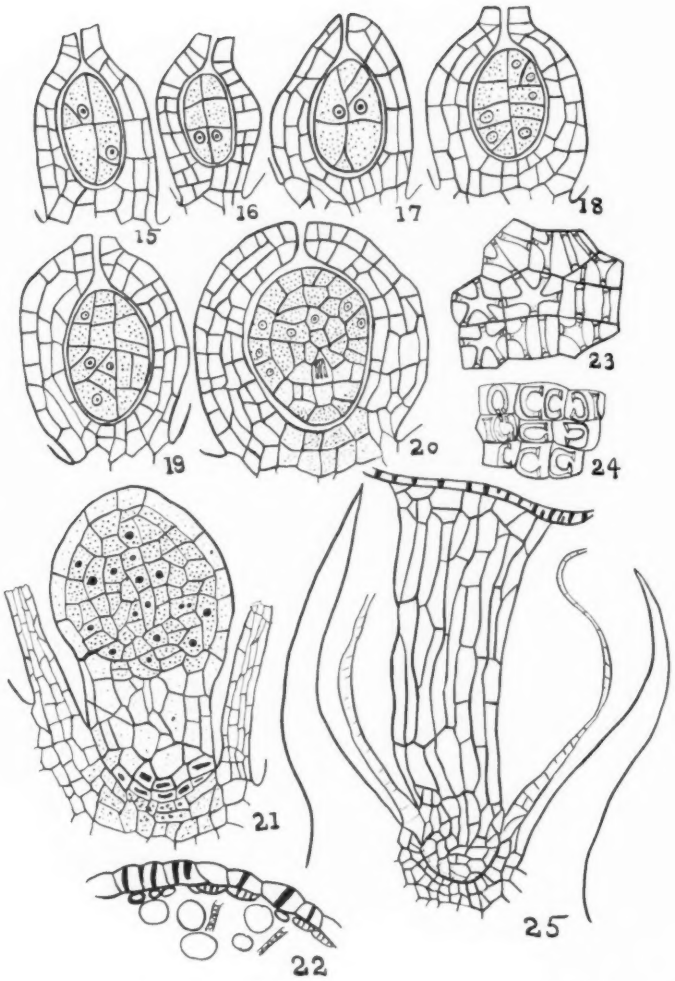
lized the receptacle remains as merely a knoblike outgrowth. Sometimes the two receptacles are united. The receptacle tissue contains air chambers filled with filaments on the dorsal surface. The cells of the roof and those forming the partitions between the air chambers contain very few chloroplasts. Each air chamber opens to the outside by well defined pores. These are of the simple type and each is surrounded by about seven cells and is not much raised. The mature receptacle is carried upward on a long stalk, although occasionally it remains sessile. The stalk has a deep furrow with numerous rhizoids. The wall of the venter of the archegonium becomes two-layered before fertilization. After fertilization the venter increases greatly in length and thickness, forming at maturity a calyptra which is four cells thick. The calyptra is ultimately ruptured near the top by elongation of the seta so as to leave it more or less two-lipped.

Material is not available for the determination of the early stages of the archegonium. The only stage found is shown in figure 10.

Actual fertilization was not observed, but the male nucleus was seen within the archegonium near the female nucleus (fig. 11). It is much more deeply stained than the female nucleus and is smaller.

### Sporophyte

After fertilization the egg divides by a transverse wall—which is slightly oblique to the longitudinal axis of the archegonium—into epibasal and hypobasal cells. After the first division the epibasal cell divides by a vertical wall (fig. 12). The hypobasal cell next divides by a vertical wall, forming a quadrant (fig. 13). Following the quadrant stage another vertical wall is formed at right angles to the first, resulting in the formation of an octant stage (fig. 14). Anticlinal walls appear soon after the octant stage. These may be formed in two diagonally opposite octants (fig. 15), or only in the four epibasal octants (fig. 16). More anticlinal walls are formed, both in the epibasal and in the hypobasal half (figs. 17, 18). After the anticlinal walls, periclinal walls appear (fig. 19). The first periclinal walls do not determine the separation of the archesporium from the capsule wall. Following the anticlinal walls, successive cell divisions produce a globular mass of cells (fig. 20). After this the growth becomes unequal, the cells in the central zone not dividing actively, with the result that an upper well developed capsule is cut




FIGS. 15–25.—Figs. 15–18, young sporophyte showing formation of anticlinal walls in epibasal and hypobasal cells. Figs. 19, 20, same showing formation of periclinal walls. Fig. 21, young sporogonium showing differentiation into foot, seta, and capsule. Fig. 22, apical cap with elater-like cells attached. Fig. 23, thickening bands on capsule wall. Fig. 24, same at base of capsule. Fig. 25, longitudinal section of sporogonium showing seta fully elongated.

off from the lower foot region. Later an outer definite layer of cells is cut off by periclinal divisions in the upper portion of the embryo which separates the sporogenous tissue from the capsule wall (fig. 21). While it is probable that the future sporogenous tissue is cut off from the capsule wall by this layer, it does not show differential staining until later.

The cells of the stalk are larger than those of the capsule and do not have much content. The cells at the base are filled with granular matter. Later on cells divide vigorously, both in the capsule and in the foot region, but especially in the sporogenous tissue of the former. The foot at a relatively early stage attains its full size, while the capsule continues to grow and becomes eventually very much larger, its diameter being twice that of the foot. The surface cells of the foot and those of the immediately surrounding tissue of the receptacle are deeply stained.

Differentiation of the central tissue of the capsule into spore mother cells and elaters takes place at a relatively late stage, and the two kinds of cells are irregularly arranged. The spore mother cells become distinguished by their size and deeply stainable character. They become spherical and, after two divisions, form four spores which remain as tetrads for a time, but when they are mature become separated from the tetrad grouping. These spores develop the characteristic compound wall consisting of three layers—endospore, exospore, and an irregular epispore. The ripe spores are tetrahedral, 52–60 $\mu$ in diameter, covered with high conical papillae on the convex surface. The elongated sterile cells mingled with the spore mother cells give rise to elaters. These are 110–140 $\mu$ in length, with three or four lax spirals, and are sometimes branched.

In the mature sporogonium the capsule wall is one cell thick, but some elater-like cells are found attached to the upper portion of the capsule. In some cases the sterile cells immediately within the capsule wall fail to separate from this at any point and consequently remain attached, so that the walls appear two or more layered (fig. 22). The apical cap is only slightly developed; however it is represented by a layer one cell thick at the apex, to which the fixed elater-like cells are attached. The cells of the capsule wall are at first thin-walled but later develop thickenings, as described by Solms-Laubach (2) for *Exormotheca pustulosa* (figs. 23, 24).

On drying, an irregular fissure is formed round the apical cap, at

its union with the capsule wall. The capsule then splits along four longitudinal lines which extend nearly to the base of the capsule, dividing the wall into four irregular valves. The apical cap remains intact, either becoming loosened and falling away or remaining attached to one of the valves. As the lines of dehiscence extend downward the valves open on the outside, exposing the mass of spores and elaters. The latter show hygroscopic movements, twisting about as they become dry and thus helping in loosening and dispersing the spores.

The seta of a nearly ripe capsule is about 0.15 mm. long and in the final stretching to elevate the capsule increases to 1 mm. (fig. 25). Elongation of the seta occurs after the capsule is practically ripe, although in exceptional cases it elongates even at the tetrad stage. The ripe capsule opens soon after being pushed out of the involucre.

## Summary

1. Vegetative reproduction in *Exormotheca tuberifera* takes place by means of tubers formed by thickening of the apex or by ventral modified shoots. Growth takes place by means of a single apical cell.

2. Development of the antheridium is like that of other Marchantiales. The receptacle contains a layer of air chambers which open on the outside by simple pores. Cell divisions occur in the venter even before fertilization, and result in the formation of a four-layered calyptra. The early development of the sporophyte shows the octant type of embryo. The apical cap is single-layered, with small elater-like cells attached to it. Dehiscence of the capsule takes place by the detachment of an apical cap and splitting up of the capsule wall into four irregular valves.

The writer expresses his deep gratitude to the late Professor S. R. KASHYAP for suggestions and criticisms during the course of this investigation.

GOVERNMENT COLLEGE
ROHTAK, PUNJAB, INDIA

## LITERATURE CITED

1. KASHYAP, S. R., Morphological and biological notes on the new and little known liverworts. II. New Phytol. **13**:308–312. 1914.
2. SOLMS-LAUBACH, H., Über *Exormotheca*, eine wenig bekannte Marchantiaceen Gattung. Bot. Zeitung. **55**:1897.

# CURRENT LITERATURE

*Plant Microtechnique.* By DONALD A. JOHANSEN. New York: McGraw-Hill Book Co., 1940. Pp. xi+523. Figs. 110.

During the last ten years numerous new methods and procedures in microtechnique have been proposed, many of which are worthy of trial and adoption. This is particularly true of certain procedures, as for example, the Feulgen nucleal reaction and the smear methods, which have proved of great value in cytological investigations. There is no single previously published source from which these techniques could be secured, and hence this volume should be of much interest and value, covering as it does in a practical and comprehensive manner the whole field of botanical microtechnique, with the exception of paleobotany and photomicrography.

The text is divided into two sections. The first describes the apparatus, reagents, killing and fixation, stains, staining procedures, special methods, whole mount methods, the glycerin method, the celloidin, paraffin, smear, cytological, and microchemical methods; and sources of materials in clear and explicit language. Nonessentials have been omitted, but each procedure has been described in sufficient detail to permit mastery in all its various phases.

The second section takes up all the plant phyla in phylogenetic sequence, giving detailed directions or suggestions so far as they are available for the specific groups in each phylum. The plants included are mainly those occurring naturally in the United States and Canada and which are readily available. Both general and specific suggestions for the collection, preservation, cultivation, and manipulation of each phylum are made at the beginning of each chapter, following which more detailed directions are given for genera and species. Numerous procedures are described for the first time, many from the author's own extensive personal experience.

An adequate bibliography is included for those who may wish to pursue a topic further and also to indicate the sources of statements, or for more detailed information concerning procedures.—J. M. BEAL.

*Liverworts of Southern Michigan.* By WILLIAM CAMPBELL STEERE. Cranbrook Institute of Science, Bloomfield Hills, Michigan, Bull. 17. Paper, $0.50; Cloth, $1.00.

Although primarily prepared for the beginner and amateur, this bulletin is so well illustrated and so ably written that the professional botanist cannot afford to be without it. The notes on each species give a brief description of the plant, its structure and reproduction, and include information relative to habitat,

abundance, and often its range in Michigan. The keys are usable and accurate. The arrangement of orders, families, and genera is conservative; it begins with the Marchantiales and ends with the Anthocerotales. Methods of collecting and preserving are amply discussed. Useful instruction in identification is presented. Necessary technical terms are defined in the glossary.

The work will be particularly useful to botanists in eastern North America, since its 58 species embrace a large proportion of the common liverworts in that area.—AARON J. SHARP.

*Flowering Shrubs of California and Their Value to the Gardener.* By LESTER ROWNTREE. Stanford University Press, 1939. Pp. xii+317. Illustrated. $3.00.

This book is a plea to horticulturists, nurserymen, and gardeners for more and better use of native California shrubs (exclusive of desert species) in home and roadside plantings. It describes the many desirable species, arranged for the most part according to native environment, together with methods for their culture. The author has drawn entirely upon her own extensive observations of the shrubs in their native habitats and upon her own experience in growing them in her California garden during the last 12 years. By her anecdotal, non-scientific though essentially accurate style, she has succeeded admirably in imparting to the average reader the same feeling of intimacy with these shrubs which she herself has acquired.—C. E. OLMSTED.

*Spore Discharge in Land Plants.* By C. T. INGOLD. New York: Oxford University Press, 1939. Pp. vi+178. $2.75.

This is a comprehensive survey of the mechanisms of spore discharge in a number of land plants, including certain of the fungi, mosses, liverworts, ferns, and club mosses. The methods involved are discussed under various headings, such as water-squirting mechanisms, discharge due to rounding-off of turgid cells, drop-excretion mechanism of basidiospore discharge, the catapult mechanism of *Sphaerobolus*, the air-gun of *Sphagnum*, mechanisms released by water-rupture, hygroscopic mechanisms, and the spiral-spring mechanism of *Frullania*. An appendix gives a classification of the seedless plants, in which is included the occurrence and type of violent spore discharge. A bibliography, followed by an index, concludes this interesting and stimulating small volume.—J. M. BEAL.

# GENERAL INDEX


## A

Absorption of soil moisture by maize roots 791

Adventitious shoots in decapitated cranberry seedlings 872

Ahmad, S., Morphological study of Exormotheca tuberifera 948

Albertson, F. W., Deterioration of grassland from stability to denudation with decrease in soil moisture 598

Allen, C. E., book reviews 508, 511

Allen, R. M., "The microscope" 719

Allison, F. E., Biochemical nitrogen fixation studies: I, II 513, 534

Alpha naphthalene acetamide, histological and physiological responses of bean plants to 204

Ammons, Nelle, "A manual of the liverworts of West Virginia" 719

Amplisiphonia, new member of the Rhodomelaceae 380

Anatomical and ontogenetic studies of flower and fruit of Fagaceae and Juglandaceae 301

Anatomy, of aerial axes of Equisetum kansanum 35; of stem of chaparral shrubs 391

Anderson, D. B., "Plant physiology" 508

Aphids, Avena coleoptile assay of ether extracts of 928

Apples, chemical determination of ethylene in emanations from 403

Arsenous acid, effect on Poa pratensis 366

Aschoff, L., et al., "Hundert Jahre Zellforschung" 508

Ashby, Helen and Eric, "German-English botanical terminology" 236

Assay of ether extracts of aphids and hosts 928

Auxin, in bean seedlings, effect of naphthalene acetic acid on 881; in marine plants 940; light stability of 726; production in isolated roots 450

Avena coleoptile assay, of ether extracts of aphids and their hosts 928; of ether extracts of nodules and roots of bean, soybean, and pea 650; light stability of auxin in 706

## B

Bain, H. F., Origin of adventitious shoots in decapitated cranberry seedlings 872

Bamford, R., Chromosome number in some tulip hybrids 482

Barkley, F. A., "Keys to the phyla of organisms including keys to the orders of the plant kingdom" 510

Beal, J. M., book reviews 955, 956

Bean, Avena coleoptile assay of ether extracts of nodules and roots 650; effect of naphthalene acid on mobile auxin in seedlings 881; effect of naphthalene acetic acid on nitrogenous and carbohydrate constituents of bean plants 688; histological and physiological responses to alpha naphthalene acetamide 204

Bellows, J. M., Jr., Chromosome number in some tulip hybrids 482

Bio-assay and source for leaf growth factors 128

Biochemical nitrogen fixation studies: I, II 513, 534

Bonner, D. M., Leaf growth hormones: I. A bio-assay and source for leaf growth factors 128

Bonner, J., Further experiments on relation of vitamin $B_1$ to growth of green plants 491

Boron and indoleacetic acid, effects on plant growth 700

Borthwick, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean 145; Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment 806; Photoperiodic responses of several varieties of soybeans 341

Browne, Isabel M. P., Anatomy of the aerial axes of Equisetum kansanum 35

Bugnicourt, F., "Les fusarium et cylindrocarpon de l'Indochine" 717

C

Calcium, effect on Poa pratensis 366

Chemical determination of ethylene in emanations from apples and pears 403

Christensen, B. E., Chemical determinations of ethylene in emanations from apples and pears 403

Chromosome number, in certain Leguminosae 51; in tulip hybrids 482

Cohen, A. L., Nutrition of the myxomycetes: I. Pure culture and two-membered culture of myxomycete plasmodia 243

Comparison of growth responses induced by naphthalene acetamide and naphthalene acetic acid 410

Conduction of rainfall by plant stems in tropical rain forest 328

Corn, physiology of lazy 68

Cowles, H. C., obituary notice 241

Cranberry seedlings, origin of adventitious shoots 872

Cribbs, J. E., Structure of fossil stem of Pityean affinity from the Reed Springs formation of Missouri 582

Culture, pure and two-membered 243

Cut flowers, keeping qualities of 501

Cytological investigations in Desmodium and Lespedeza 839; studies of Lilium tigrinum 550

D

Darrow, R. A., Effects of soil temperature, pH, and nitrogen nutrition on development of Poa pratensis 109

Davis, C. H., Absorption of soil moisture by maize roots 791

Density of stomata and oil glands in rind of orange 862

Desmodium, cytological investigations 839

Drought, effect on grassland 598

E

Eaton, F. M., Interrelations in effects of boron and indoleacetic acid on plant growth 700

Effect of naphthalene acetic acid, on mobile auxin in bean seedlings 881; and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants 688

Effect, of potato extracts on growth 912; of soil temperature, pH, and nitrogen nutrition on development of Poa pratensis 109; of temperature, calcium, and arsenous acid on Poa pratensis 366; of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean 145

Effectiveness of growth substances on parthenocarpy in holly 226

Eggers, Virginia, Avena coleoptile assay of ether extracts of aphids and their hosts 928; Avena coleoptile assay of ether extracts of nodules and roots of bean, soybean, and pea 650

Embryo and fruit of peach, growth affected by ringing and defoliation of branches 818

Emerson, S., Growth of incompatible pollen tubes in Oenothera organensis 890

Engard, C. J., Translocation of nitrogenous substances in the Cuthbert raspberry 1

Environmental factors, influence on photoperiodic induction 168

Equisetum kansanum, anatomy of aerial axes 35

Ethylene, chemical determination of in emanations from apples and pears 403

Evidence for auxin production in isolated roots 450

Exormotheca tuberifera, morphological study 948

F

Fagaceae, ontogenetic and anatomical studies of flower and fruit 301

Fassett, N. C., "The leguminous plants of Wisconsin" 510

Feulgen reaction, use of in cytology 276

Fixatives, effect on Feulgen reaction in cytology 276

Floral initiation in Biloxi soybeans receiving photoperiodic treatment 806

Fluorescent lamps for growing plants 715

Fossil stem of Pityean affinity 582

Frey-Wyssling, A., "Submikroskopische Morphologie des Protoplasmas und seiner Derivate" 718

## G

Gardner, F. E., Effectiveness of several growth substances on parthenocarpy in holly 226

Germination and vegetative stages of gametophytes of Hymenophyllum and Trichomanes 759

Gerner, Gladys, Fluorescent lamps as source of light for growing plants 715

Grassland, deterioration of 598

Greene, J., Further experiments on relation of vitamin $B_1$ to growth of green plants 491

Grout, A. J., "Moss flora of North America north of Mexico" 509

Growth and development of embryo and fruit of peach affected by ringing and defoliation of branches 818

Growth, effect of potato extracts on 912; of green plants, experiments on relation of vitamin $B_1$ 491; of incompatible pollen tubes in Oenothera organensis 890

Growth inhibitor and inhibition 91

Growth responses, induced in plants by naphthalene acetic acid 410; of soybeans to phosphate and nitrate in nutrient solution 637

Growth substances, and gametic reproduction by Phycomyces 428; effectiveness on parthenocarpy in holly 226

## H

Haagen-Smit, A. J., Leaf growth hormones: I. A bio-assay and source for leaf growth factors 128

Hamner, C. L., Growth responses of Biloxi soybeans to variation in relative concentrations of phosphate and nitrate in the nutrient solution 637

Hamner, K. C., Effect of potato extracts on growth of Phycomyces 912; Interrelation of light and darkness in photoperiodic induction 658; Localization of photoperiodic perception in Helianthus tuberosus 81

Hansen, E., Chemical determination of ethylene in the emanations from apples and pears 403

Helianthus tuberosus, localization of photoperiodic perception 81

Hillary, B. B., Use of the Feulgen reaction in cytology: I. Effect of fixatives on the reaction 276

Hirsch, G. C., "Form- und Stoffwechsel der Golgi-Körper" 511

Histological responses of bean plants to alpha naphthalene acetamide 204

Hollenberg, G. J., A morphological study of Amplisiphonia, a new member of the Rhodomelaceae 380

Holly, Effect of growth substances on parthenocarpy 226

Hoover, S. R., Biochemical nitrogen fixation studies: I, II 513, 534

Hormones, leaf growth, bio-assay and source for 128

Hymenophyllum, spore germination and vegetative stages of gametophytes 759

## I

Incompatible pollen tubes, growth of in Oenothera organensis 890

Indoleacetic acid and boron, effects on plant growth 700

Ingold, C. T., "Spore discharge in land plants" 956

Interrelation of light and darkness in photoperiodic induction 658

Interrelations in effects of boron and indoleacetic acid on plant growth 700

## J

Johansen, D. A., "Plant microtechnique" 955

Johnson, M. A., Structure of the shoot apex in Zamia 189

Jones, S. G., "Introduction to floral mechanism" 237

Juglandaceae, ontogenetic and anatomical studies of flower and fruit 301

## K

Keeping qualities of cut flowers 501

Klotz, L. J., Density of stomata and oil glands and incidence of water spot in rind of Washington navel orange 862

Knudson, L., Permanent changes of chloroplasts induced by X rays in gametophyte of Polypodium aureum 721

Kraus, E. J., Histological and physiological responses of bean plants to alpha naphthalene acetamide 204; Obituary notice of Henry Chandler Cowles 241; Obituary notice of Adolf Carl Noé 231; book reviews 236, 237, 510, 719

Kühner, R., "Le genre Mycena. Étude et systématique des espèces d'Europe et d'Amérique du Nord" 235

L

Langdon, LaDema M., Ontogenetic and anatomical studies of the flower and fruit of the Fagaceae and Juglandaceae 301

Layering in eastern white pine 505

Lee, F. A., Growth and development of the embryo and fruit of the peach as affected by ringing and defoliation of the branches 818

Legume nodules and oxygen supply 513, 534

Leguminosae, chromosome numbers in 51

Lespedeza, cytological investigations 839

Light, fluorescent lamps for growing plants 715

Light stability of auxin 706

Lilium tigrinum, cytological studies 550

Link, G. K. K., Avena coleoptile assay of ether extracts of aphids and their hosts 928; Avena coleoptile assay of ether extracts of nodules and roots of bean, soybean, and pea 650

Localization of photoperiodic perception in Helianthus tuberosus 81

Long, E. M., Localization of photoperiodic perception in Helianthus tuberosus 81; Photoperiodic induction as influenced by environmental factors 168

Ludwig, C. A., Biochemical nitrogen fixation studies: I, II 513, 534

Lutz, H. J., Layering in eastern white pine 505

M

Marth, P. C., Effectiveness of several growth substances on parthenocarpy in holly 225

Martin, G. W., book reviews 235, 237, 717

Meyer, B. S., "Plant physiology" 508

Minor, F. W., Biochemical nitrogen fixation studies: I, II 513, 534

Mitchell, J. W., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid 410; Effect of naphthalene acetic acid and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants 688; Histological and physiological responses of bean plants to alpha naphthalene acetamide 204

Morphological study of Exormotheca tuberifera 948

Moulton, J. E., Avena coleoptile assay of ether extracts of aphids and their hosts 928

Myrciaria cauliflora, polyembryony in 233

Myxomycetes, nutrition of 243

N

Naphthalene acetamide, comparative growth effects with naphthalene acetic acid 410; histological and physiological responses of bean plants to 204

Naphthalene acetic acid, effect on mobile auxin in bean seedlings 881; effect on nitrogenous and carbohydrate constituents of bean plants 688

Naumov, N. A., "Clés des Mucorinées" 237

Naylor, A. W., Effects of temperature, calcium, and arsenous acid on seedlings of Poa pratensis 366; Fluorescent lamps as source of light for growing plants 715

Neff, M. S., Color and keeping qualities of cut flowers 501

New genus of the Rhodomelaceae 380

Nitrogen fixation studies: I, II 513, 534

Nitrogen nutrition, temperature, and pH effects on development of Poa pratensis 109

Nitrogenous substances in Cuthbert raspberry 1

Noé, A. C., obituary notice 231

Nuclei, size of in shoot of Ricinus communis 625

Nutrient solution 637

Nutrition of the myxomycetes 243

O

Oenothera organensis, growth of incompatible pollen tubes in 890

Ogura, Y., "Anatomie der Vegetationsorgane der Pteridophyten" 509

Olmsted, C. E., book reviews 235, 956

Ontogenetic and anatomical studies of flower and fruit of Fagaceae and Juglandaceae 301

Orange, density of stomata and oil glands 862

Origin of adventitious shoots in decapitated cranberry seedlings 872

Oxygen supply within the nodule 513, 534

P

Parker, M. W., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean 145; Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment 806; Photoperiodic responses of several varieties of soybeans 341

Parthenocarpy in holly, effectiveness of growth substances on 226

Pea, Avena coleoptile assay of ether extracts of nodules and roots 650

Peach, embryo and fruit affected by ringing and defoliation of branches 818

Pears, chemical determination of ethylene in emanations from 403

Permanent changes of chloroplasts induced by X rays 721

Photoperiodic induction, and interrelation of light and darkness 658; as influenced by environmental factors 168; effect of temperature variation upon initiation of flower primordia in Biloxi soybean 145

Photoperiodic, perception in Helianthus tuberosus 81; responses of soybeans 341; treatment affecting floral initiation in Biloxi soybeans 806

Phycomyces, effect of potato extracts on growth of 912; growth substances and gametic reproduction by 428

Physiological responses of bean plants to alpha naphthalene acetamide 204

Physiology of lazy corn 68

Pine, layering in eastern white 505

Plant growth inhibitor and growth inhibition 91

Plastids of Polypodium, affected by X rays 721

Poa pratensis, effects of soil temperature, pH, and nitrogen nutrition on 109; effects of temperature, calcium, and arsenous acid on 366

Polyembryony in Myrciaria cauliflora 233

Polypodium, X rays in gametophyte of 721

Potato extracts, effect on growth of Phycomyces 912

R

Rainfall, conduction by plant stems in tropical rain forest 328

Raspberry, translocation of nitrogenous substances in 1

Respiration of nodules and roots, including non-legume roots 534

Reynard, G. B., Chromosome number in some tulip hybrids 482

Ricinus communis, size of nuclei in shoot 625

Robbins, W. J., Effect of potato extracts on growth of Phycomyces 912; Growth substances and gametic reproduction by Phycomyces 428

Rowntree, Lester, "Flowering shrubs of California and their value to the gardener" 956

S

Scott, Flora M., Size of nuclei in shoot of Ricinus communis 625

Sex expression in willows 851

Shafer, J., Jr., Physiology of lazy corn 68

Sharp, A. J., book review 955

Shull, C. A., book reviews 238, 508, 717

Smith, E. C., Sex expression in willows 851

Soil moisture 598; absorption by maize roots 791

Soybean, Avena coleoptile assay of ether extracts of nodules and roots 650; effect of variation in temperature during photoperiodic induction upon initiation of flower primordia 145; floral initiation of 806; growth responses to phosphate and nitrate 637; photoperiodic responses 341

Spore germination and vegetative stages of gametophytes of Hymenophyllum and Trichomanes 759

Steere, W. C., "Liverworts of southern Michigan" 955

Stem anatomy of chaparral shrubs 391

Stewart, W. S., book review 718; Effect of naphthalene acetic acid on mobile auxin in bean seedlings 881; Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid 410; Light stability of auxin in Avena coleoptiles 706; Plant growth inhibitor and plant growth inhibition 91

Stokey, Alma G., Spore germination and vegetative stages of gametophytes of Hymenophyllum and Trichomanes 759
Stomata and oil glands in rind of orange 862
Structure of fossil stem of Pityean affinity 582; of shoot apex in Zamia 189

T

Tansley, A. G., "The British Islands and their vegetation" 235
Temperature, effect of variation during photoperiodic induction upon initiation of flower primordia in Biloxi soybean 145; effect on Poa pratensis 109, 366
Translocation of nitrogenous substances in Cuthbert raspberry 1
Traub, H. P., Polyembryony in Myrciaria cauliflora 233
Trichomanes, spore germination and vegetative stages of gametophytes 759
Tropical rain forest, conduction of rainfall by plant stems 328
Tukey, H. B., Growth and development of the embryo and fruit of the peach as affected by ringing and defoliation of branches 818
Tulip hybrids, chromosome number in 482
Turner, W. L., "Growing plants in nutrient solutions" 717
Turrell, F. M., Density of stomata and oil glands and incidence of water spot in rind of Washington navel orange 862

U

Use of the Feulgen reaction in cytology 276

V

Van Overbeek, J., Auxin in marine plants: II 940; Evidence of auxin production in isolated roots growing in vitro 450
Vernalization of Marquis wheat and other spring cereals 457
Voth, P. D., book reviews 509, 510, 719; Conduction of rainfall by plant stems in tropical rain forest 328

W

Water spot, in rind of orange 862
Watkins, Katherine S., Stem anatomy of chaparral shrubs 391
Weaver, J. E., Deterioration of grassland from stability to denudation with decrease in soil moisture 598
Went, F. W., Leaf growth hormones: I. A bio-assay and source for leaf growth factors 128; Light stability of auxin in Avena coleoptiles 706
Westfall, J. J., Cytological studies of Lilium tigrinum 550
Westveld, R. H., "Applied silviculture in the United States" 510
Wheat, vernalization of 457
Willis, L. G., "Bibliography of references to the literature on the minor elements and their relation to plant and animal nutrition" 238
Willows, sex expression in 851
Wipf, Louise, Chromosome numbers in root nodules and root tips of certain Leguminosae 51
Wort, D. J., Vernalization of Marquis wheat and other spring cereals 457

X

X rays in gametophyte of Polypodium 721

Y

Young, J. O., Cytological investigations in Desmodium and Lespedeza 839

Z

Zamia, structure of shoot apex 189